REVIEW OF LITERATURE

Helminth Infections

Helminths are commonly known as parasitic worms or sometimes referred as intestinal worms and are large multi-cellular organisms which when mature can generally be seen with the naked eye. They live on feeding on living hosts, receiving nourishment and protection while disrupting their hosts' nutrient absorption, causing weakness and disease. The adult worms live inside the digestive tract or intravascular tissues and cannot multiply in humans. Helminthes are commonly classified into three major groups namely, cestodes or commonly known as tapeworms, trematodes or commonly known as flukes and nematodes which are commonly known as roundworms. The roundworms or nematodes in their adult form can reside in the gastrointestinal tract, blood, lymphatic system or subcutaneous tissues. Alternatively, the immature (larval) states can cause disease through their infection of various body tissues. (CDC, 2014). The three most commonly causative nematode infections are hookworm infection, lymphatic filariasis and strongyloidiasis. Hookworm infection and strongyloidiasis are caused by intestinal worms, where the site of infection is the gut or lumen, whereas lymphatic filariasis is caused by filarial worms, where the site of infection is in the lymphatics.

Hookworm infection

Hookworm is a soil-transmitted helminth and is one of the most common roundworm of humans that reside in the small intestine. Infection is caused by the nematode parasites *Necator americanus* and *Ancylostoma duodenale*. An estimated 576-740 million people in the world are infested with hookworm. Hookworm infections are transmitted when the eggs are passed in the faeces of an infected person. If the infected
person defecates under improper sanitary conditions outside or if the faeces of an infected person are used as fertilizer, eggs are deposited on soil. They can then mature and hatch, releasing larvae (immature worms). The larvae mature into a form that can penetrate the skin of humans.

**Life cycle of hookworm infection**

The life cycles of hookworms are considered excellent examples of direct transmission. The simplicity in the life cycle and poor sanitation contributes to the endemicity of hookworm infection and the rapid re-infection after chemotherapy. (Gunawardena et al., 2005).

---

**Figure I: Life cycle of hookworm infection.** Adapted from [http://www.cdc.gov/parasites/hookworm/biology.html](http://www.cdc.gov/parasites/hookworm/biology.html)
Eggs are evacuated along with human faeces which are deposited in the soil. The eggs hatch after 24–48 h, under proper humidity and ambient temperature. The first stage or L1 are referred to as rhabditiform larvae and after a brief period moult into the L2 stage. Both larval stages are free-living, meaning they are not dependent on a host for food. They live by feeding on organic debris and bacteria present in the soil. The L2 later develop into the infective L3 stage, which measure approximately 600 μm in length. Unlike the previous stages, the L3 are in a state of arrested development and do not feed; although, they can survive for weeks in the environment using energy accumulated during the previous two larval stages. (Brooker et al., 2004). To increase the chances of survival and an encounter with the host, the L3 seek higher ground to be more exposed, finding their target through a combination of positive thermo tropism (movement towards higher temperatures) and positive thigmotropism (movement towards an object in response to touch or contact stimuli). After contact with the human host, L3 penetrates through the skin, usually through hair follicles, and make their way to the capillaries, migrating through the vessels to reach the heart and then later passing into the lungs. Once in the lungs, L3 larvae ascend to the trachea, where they are coughed up and swallowed, starting their migration through the gastro-intestinal tract. The transformation into L4 larvae occurs in the lumen, where they finally mature into the adult form. (Hawdon and Hotez, 1996). The prepatent period (i.e. the time taken by the larvae to penetrate the skin and reach the intestine to become sexually mature egg-laying adult worms) for both human hookworm species lasts between 5 and 8 weeks. Mature males and females copulate in the lumen and generate eggs, which pass out with the faeces into the environment, restarting the life cycle. Female adult worms can
produce up to 1000 eggs per day (Brooker, Bethony and Hotez, 2004), (Diemert Bethony and Hotez, 2008).

**Symptoms and diagnosis of hookworm infection**

Hookworm infection is mainly acquired by walking barefoot on contaminated soil. High-intensity hookworm infections occur among both school-age children and adults, unlike the soil-transmitted helminths like *Ascaris* and whipworm. The most serious effects of hookworm infection are the development of anaemia and protein deficiency caused by blood loss at the site of the intestinal attachment of the adult worms. When children are continuously infected by many worms, the loss of iron and protein can retard growth and mental development. Most people infected with hookworms do not show any symptoms. Some persons who are infected for the first time have gastrointestinal symptoms. The most serious effects of hookworm infection are blood loss leading to anaemia, in addition to protein loss. The standard method for diagnosing the presence of hookworm is by identifying hookworm eggs in a stool sample using a microscope. Because eggs may be difficult to find in light infections, a concentration procedure is recommended.

**Treatment of hookworm infection**

Anthelminthic medications (drugs that rid the body of parasitic worms), such as albendazole and mebendazole, are the drugs of choice for treatment of hookworm infections. Infections are generally treated for 1-3 days. The recommended medications are effective and appear to have few side effects. Iron supplements may also be prescribed if the infected person has anaemia.
Immune responses to hookworm infection

The most studied aspect of the human immune response comes from epidemiological studies in hookworm-endemic areas, most of which initially centred on the systemic and specific humoral response to infection. Antibody responses to crude antigen extracts could be observed for all five isotypes: IgA, IgD, IgE, IgG and IgM (Carr and Pritchard, 1987), (Loukas and Prociv, 2001). Some of these antibody responses correlated with different aspects of the infection: for example, IgG to adult ES (excretory-secretory) products was shown to correlate with parasite burden measured by EPG (eggs per gram) and titres of the antibody response decreased with the age of the host (Quinnell et al., 1995). Moreover, the majority of the studies conducted in endemic areas occurred in either Papua New Guinea focusing on the antibody response in relationship to the intensity of the infection (Quinnell et al., 2004), (Pritchard et al., 1992), (Pritchard et al., 1995), (Quinnell et al., 1995), (Pritchard et al., 2007), (Ricci et al., 2011) and Brazil focusing more on the cell-mediated response to hookworm infection (Geiger et al., 2004), (Geiger et al., 2007), (Geiger et al., 2011). The Papua New Guinea study demonstrated an age-specific humoral response with a significant correlation between IgG against crude L3 antigen extract and age, a significant negative correlation between IgG against adult ES products and pre-treatment burdens in individuals aged 33–48 years and a significant negative correlation between anti-ES IgG and IgE in this same age group (Quinnell et al., 1995). In another study conducted in this same area, total IgE levels during hookworm infection increased compared with pre-infection levels and were significantly correlated with an increase in pre-treatment worm burden (Quinnell et al., 2004). Another study also showed that levels of total IgE and eosinophils were negatively correlated with the weight and fecundity of the
worms, that is, the higher the levels of total IgE and eosinophils, the lower the weight and fecundity of worms recovered from the hosts after treatment (Pritchard et al., 1995). This group also showed that the levels of IFN-γ were negatively correlated with egg burden, that is, the higher the level of IFN-γ, the lower the egg burden (Quinnell et al., 2004). The suggestion that this cytokine might act as a protective mechanism against the infection is still unclear (Quinnell et al., 1995), (Quinnell et al., 2004). Pritchard et al. also studied basophil competence during hookworm infection in individuals in Papua New Guinea. In this study, they observed the presence of IgG anti-IgE auto antibodies, an increase in eosinophils and no change in the number of basophils between infected and uninfected individuals. In an in vitro assay using basophils from infected individuals, they observed that anti-human IgE and normal and heat-inactivated ES from Necator-induced histamine release from the basophils in a dose-dependent manner. In contrast, basophils from uninfected volunteers released significantly less histamine after exposure to anti-IgE. There was also a difference in the levels of total and ES-specific IgE between infected and uninfected volunteers. Therefore, they concluded that basophils remain competent in hookworm-infected individuals from an endemic area, suggesting that hookworm down-regulation of immune responses is not due to the blockade of the IgE receptor FcεRI (Pritchard et al., 2007). The studies conducted in endemic areas of Brazil are from an area where co-endemicity with other parasitic helminths is common, and therefore, we focus on the results obtained only from individuals mono-infected with hookworm. In a study conducted in school-aged children, the comparison of unstimulated PBMCs of cured children (verified to be cured during the study using the Kato-Katz technique) and hookworm-infected children (those
who remained positive after treatment) yielded higher baseline levels of TNF-α than negative endemic controls (proven to be negative during 6 months prior to enrolment in the study). Interestingly, the PBMCs stimulated with hookworm AE (soluble somatic antigen extracts were prepared from third-stage larvae (L3) and adult worms (AE) of *Ancylostoma caninum*) of the egg-negative control group showed higher levels of IL-12, IFN-γ, IL-5 and IL-13 than the hookworm-infected children. The only cytokine level observed to be higher in the AE-stimulated PBMCs of infected children compared with egg-negative controls was IL-10 (Geiger et al., 2004).

In another study conducted in adults and children from a *Necator*-endemic area in Brazil, only individuals with hookworm infection, as determined by the presence of eggs in faeces, were included in the study and stratified by age. The study showed correlations between egg burden and cytokines produced by stimulated PBMCs of infected people (Geiger et al., 2011). The egg burden in people mono-infected with *N. americanus* was negatively correlated with IL-10 production from PBMCs cultured with the crude antigen extracts L3, AE or ES. Moreover, TNF-α levels produced by these cells in culture with ES also correlated negatively with egg burden (Geiger et al., 2011). This agrees with a previously published study on cellular responses during *N. americanus* infection, which demonstrated that hookworm-infected individuals had less T cells (CD3 and CD4) and B cells (CD19) than the non-infected control group, as measured through lymphocyte phenotyping of unstimulated whole blood samples (Geiger et al., 2007). Although the mean percentages of CD4 were lower in *Necator*-infected individuals compared with egg-negative individuals, the levels of activated CD4 (HLA-DR+) were elevated. The number of activated T cells (CD3+CD69+, CD8+HLA-DR+) and B cells (CD19+CD27+) was also higher than in the
non-infected individuals. In keeping with other studies, the cytokine response to the crude *N. americanus* antigen extracts was mixed. This mixed response (Th1/Th2) was lower in infected individuals than in egg-negative individuals, especially in response to AE and ES, indicating a modulatory effect of *N. americanus* infection. This study also showed a positive correlation between the levels of IFN-γ and IL-5 produced by PBMCs stimulated with L3 that contradicts the idea that IFN-γ has an antagonistic effect on Th2 responses. Contrary to the previous studies from this same group, the production of IL-10 from PBMCs stimulated with AE, ES and L3 was higher in egg-negative compared with infected individuals (Geiger et al., 2007). This discrepancy could be due to the fact that in the previous, the cured and infected individuals were treated prior to blood collection studies (Geiger et al., 2004).

Another study in Brazil evaluated the role of regulatory T cells (Tregs) during human hookworm infection. Infected individuals showed greater levels of Tregs identified using CD4, CD25 and FoxP3 as markers than non-infected controls (Ricci et al., 2011). These Tregs also showed higher expression of CTLA4 and GITR surface receptors as well as IL-10, TGF-β and IL-17. Interestingly, the only hookworm crude antigen extract that showed regulatory capacity through a decrease in the production of IL-17 was AE. Finally, after the depletion of CD4+CD25+ cells, proliferation of CD4 was increased in cells cultured with AE and ES and proliferation of CD8 was increased in cells cultured with ES, showing that the Tregs were functional (Ricci et al., 2011).

In a study conducted in Togo, Africa, the immunological parameters of hookworm were measured using umbilical cord cells from neonates and PBMCs from their mothers who were infected with hookworm. The results showed that cells from both of these sites
naturally produced Th1 (IL-2, IFN-γ and IL-12) as well as Th2 and modulatory cytokines (IL-5 and IL-10, respectively). When umbilical cord cells from neonates and PBMCs from mothers were stimulated with crude AE antigen extract, both cell sources produced similar levels of IL-2, IL-5 and IL-10, but the umbilical cells produced more IL-12 and less IFN-γ than the PBMCs from the mothers (Pit et al., 2000). These results suggest that offspring of infected mothers were sensitized in the uterus. However, this study did not obtain data on the immunological response of these children after birth or determine whether their response differed from that seen in hookworm-infected children whose mother’s where not infected during pregnancy. Another study conducted in adults from a hookworm-endemic area in Zimbabwe assessed the relationship between the production of *N. americanus* AE-specific antibodies and the age, sex and infections status of the participants. Serum levels of AE-specific IgG, IgG2, IgG3, IgG4 and IgE increased as the eggs in faeces increased, while the level of AE-specific IgG4 also positively correlated with worm load (measured as the number of worms expelled after treatment). The relationship between age and isotype responses also showed a positive correlation with serum AE-specific IgG, IgG3, IgG4 and IgA (Palmer et al., 1996).

In summary, natural hookworm infection is an uncontrolled infection (with respect to the inoculums) that tends to be chronic in nature, often increasing in intensity with the age of the infected individual (Bethony et al., 2002). Studies conducted in naturally infected populations show that individuals present the following antibodies in response to hookworm infection: L3-specific IgG and IgM, ES-specific IgG, IgM and IgE, AE-specific IgG, IgG2, IgG2, IgG4 and IgE, and increased levels of total IgE. Levels of total IgE and AE-specific IgG4 are positively correlated with worm burden, while total IgE is negatively
correlated with the weight and fecundity of the worms. AE-specific IgG, IgG2, IgG3, IgG4 and IgE are positively correlated with EPG of faeces. The age of the individuals is also positively correlated with the levels of AE-specific IgG, IgG3, IgG4 and IgA. Infected individuals also present eosinophilia and, although they do not present basophilia, their basophils remain competent despite continuous contact with the parasite. The increase in eosinophils is negatively correlated with the weight and fecundity of the worms.

However, these studies show conflicting results for the production of cytokines, probably due to differing immunological methods and study populations. Higher levels of TNF-α and lower levels of IL-12, IFN-γ, IL-5 and IL-13 were observed in PBMCs stimulated with AE of hookworm-infected compared with egg-negative individuals. When PBMCs from infected individuals were cultured with L3, there was a positive correlation between IFN-γ and IL-5, although there was no difference between the groups. The production of IL-10 from PBMCs stimulated with AE, ES and L3, on the other hand, was shown to be lower in infected than in egg-negative individuals in one study and higher in another study where the individuals were treated prior to blood collection. Nonetheless, the IL-10 produced in the presence of L3, AE and ES is negatively correlated with the amount of eggs in faeces, which is also true for IFN-γ. Also, infected participants had a greater number of functional Tregs than uninfected individuals. Finally, during natural human infections with hookworm, a mixed Th1/Th2 response is observed (Gaze et al., 2014).

Experimental human infections have provided major contributions to our understanding of primary hookworm infection. The immunology of this infection is a strong eosinophil presence during the first days after L3 skin penetration, followed by a strong pro-inflammatory response, with the presence of IFN-γ, IL-2, IL-15 and IL-22 but not IL-17A
or IL-23 cytokines. The source of the Th1 cytokine IFN-γ is thought to be from innate cells (e.g. NK cells). Experimental hookworm infections also present a Th2 and modulatory responses with the presence of IL-5, IL-13, IL-9 and IL-10 cytokines, even when the number of infective larvae is low (15 L3). On the other hand, natural infections show a mixed Th1/Th2 response in the production of cytokines and chemokines, a strong T and B cell activation, and a strong humoral response with the presence of IgG, IgM and IgE antibodies specific to all crude antigen extracts from different stages of the life cycle. Therefore, both types of studies in humans, natural and experimental, contribute to different aspects to the immunology of hookworm infection. Experimental infections contribute to the understanding of a primary infection, and studies conducted in endemic areas can add information on chronic infections. Thus, by comparing both strategies, more knowledge is obtained to understand the immunological events that occur during hookworm infection, which may also lead to better intervention strategies to control this highly prevalent neglected tropical disease (Gaze et al., 2014)

**Lymphatic filariasis**

Unlike hookworm infection, lymphatic filariasis is caused by lymphatic tissue dwelling worms such as *Wuchereria bancrofti, Brugia malayi* and *Brugia timori*. All three parasites are transmitted by the bites of infective mosquitoes and share similar life cycles in humans with the adult worms living in the afferent lymphatic vessels while the microfilariae, are present in systemic circulation and are readily available to infect the mosquitoes during a blood meal. Though typically not fatal, lymphatic filariasis is responsible for considerable suffering, deformity and disability and is the second leading parasitic cause of disability with DALYs (disability-adjusted life years) estimated to be
5.549 million. Bancroftian filariasis, caused by *W. bancrofti*, is responsible for 90% of those with lymphatic filariasis and is found throughout the tropics and some sub-tropical areas (Fenwick, 2012). The rest are caused by Brugian parasites that have a more restricted geographical distribution. Lymphatic filariasis is a global health problem. At the present time (2012), the World Health Organization estimates that over 1.25 billion people are at risk in 72 countries and territories. Approximately 120 million people already have been infected with lymphatic filariasis and over 40 million are seriously incapacitated or disfigured by the disease. Clinical disease is manifested primarily as acute and chronic lymphedema, which may lead to elephantiasis in men and women and to the formation of hydroceles in men.

**Life cycle of lymphatic filariasis**

All human filarial nematodes have a complex life cycle involving an insect vector, with Wuchereria and Brugia being transmitted by mosquitoes. Infection begins with the deposition of infectious-stage larvae or L3 larvae in the skin during a mosquito bite. The larvae then crawl in through the puncture wound and enter into the lymphatics and lymph nodes. They undergo a process of molting and development to form L4 larvae and then adult worms. The adult worms reside within the lymphatics and lymph nodes and following mating release live progeny called microfilariae (mf), which circulate in the bloodstream. These microfilariae can then be ingested by a mosquito during a blood meal, where in they undergo development to form L2 and finally L3 larvae and the life-cycle continues. The complex life-cycle engenders a complicated host immune response, and it is this complexity of the host-parasite interaction that is thought to underlie the varied clinical
manifestations of lymphatic filariasis. (Centres for Disease Control: Global Health – Division of Parasitic Diseases and Malaria, November 2010)

Figure II: Life cycle of *Wuchereria bancrofti* infection. Adapted from [http://www.cdc.gov/parasites/lymphaticfilariasis/biology_w_bancrofti.html](http://www.cdc.gov/parasites/lymphaticfilariasis/biology_w_bancrofti.html)

**Immune responses to lymphatic filariasis**

The most important host immune response to filarial parasites in both mice and humans was the T helper 2 (Th2) type of response which involves the production of cytokines – IL-4, IL-5, IL-9, IL-10 and IL-13, the immunoglobulin G isotypes – IgG1, IgG4 (in humans) and IgE, and skewed populations of eosinophils and alternatively activated macrophages (Allen and Maizels, 2011). Initially the T cells would interact with a variety of host cell types including dendritic cells and macrophages and induce Th2
responses (Allen and Maizels, 2011). These parasite specific Th2 responses were modulated by both adaptive and natural regulatory T cells, alternatively activated macrophages, and eosinophils (Babu and Nutman, 2012). The main characteristic of a chronic filarial infection appears to be the presence of a modified Th2 response and an IL-10 dominated regulatory environment (Babu and Nutman, 2012).

It is well known that the T cells are the key players in immunity to filarial infections. Both nude mice (that lack T cells) (Suswillo et al., 1980), (Vincent, Sodeman & Winters, 1980) and SCID or Rag-deficient mice (that lack both T and B cells) (Nelson et al., 1991), (Babu et al., 1999) were susceptible to Brugian infection that indicated that T cells were absolutely critical for parasite elimination. In addition, it was also shown that protective immunity to filarial infections in mice was dependent primarily on Th2 responses. Thus, mice which lacked IL-4, IL-4R or Stat6 (all deficient in Th2 responses) were all susceptible to Brugian infection (Babu et al., 2000), (Spencer et al., 2001). Interestingly, it was also found that IFNγ also appeared to play a role in protection against infection because mice lacking IFNγ exhibited impairment parasitic elimination (Babu et al., 2000). Therefore, protective immunity to filarial infections required coordination of both Th1 and Th2 responses.
Figure III: Immunology of lymphatic filariasis. Regulation of immune responses in filarial infection. The complex outcome of the interaction between the filarial parasite and the host immune system determines the immunological outcomes including (a) protection against infection; (b) parasite-specific T-cell hyporesponsiveness and alteration of APC function; (c) chronic infection; (d) protection against pathology and (e) anti-inflammatory bystander suppression.

One of the most consistent findings in filarial infections was the elevated level of IgE which was observed following L3 exposure (Hussain et al., 1981). Most of the IgE produced was polyclonal IgE indicating a non-antigen-specific induction of IgE-producing B cells. (Hussain & Ottesen, 1985) It was also explained that, these IgE antibodies remained detectable many years after the infection has been treated indicating the presence of long-lived memory B cells or plasma cells in filarial infections (Mitre & Nutman, 2006)
IgE production both in mice and humans was absolutely dependent on IL-4 or IL-13. Other isotypes that were commonly elevated in chronically filarial-infected humans are IgG4 and IgG1, the former being most dependent on both IL-4 and IL-10 (Nutman & Kumaraswami, 2001). Thus, in vivo data from mice deficient in IgE showed increased worm burdens with *B. malayi* indicating an important role for IgE in host defense (Spencer LA et al., 2003). In the lymphatics and lymph nodes as well as in the circulation, it was shown that filarial parasites were susceptible to attack by a range of host innate effector cells, including macrophages, eosinophils and neutrophils. The ability of these cells to kill the parasites was often dependent on either IgE or IgM and also complement.

Dendritic cells are professional antigen-presenting cells (APCs) which have shown to play an essential role in presenting antigen to T cells to initiate immune responses. It has been shown that differentiation and maturation of DC in the presence of filarial antigens in vitro can stimulate Th2 responses by down modulating IL-12 production (Semnani et al., 2003). In addition, live parasites have also been shown to destroy human dendritic cells and diminish their capacity to activate CD4+ T cells (Semnani et al., 2003). Macrophages were known to be the other important class of antigen-presenting cells that could serve as protective effector cells in bacterial and protozoan infections by their production of nitric oxide and other mediators. A special class of macrophages was known to be induced in filarial infections, which were characterized by their preferential expression of the enzyme arginase, instead of nitric oxide due to increased activation of *arginase-1* by IL-4 and IL-13 (Loke et al., 2000). These macrophages were termed as alternatively activated macrophages and had a very specific gene expression profile, that had the ability to up regulate markers
including arginase-1, chitinase 3-like proteins 3 and 4 (also known as YM1 and YM2, respectively) and resistin-like molecule-α (RELMα) (Nair et al., 2005) These alternatively activated macrophages were known to play an important role in wound healing and helped limit tissue immunopathology (Allen & Wynn, 2011). By virtue of expression of regulatory molecules such as IL-10, TGFβ and programmed cell death 1 ligand 2 (PDL2), these macrophages might be considered to play a predominant regulatory role in filarial infections (Allen & Wynn, 2011). However, these filarial-induced macrophages appeared to have the ability to expand locally and were less dependent on influx of monocytes from the bloodstream to perform their functions (Jenkins et al., 2011). While filarial infection does induce expression of these cells in humans, early interaction of parasites or parasite antigens lead to a predominantly pro-inflammatory response with expression of mainly pro-inflammatory cytokines that included TNFα, IL-6 and IL-1β, and genes involved in inflammation and adhesion (Chaussabel et al., 2003).

It is well understood that blood eosinophilia is characteristic of filarial infection and was mediated by IL-5 (probably concurrent with IL-3 and GM-CSF) (Klion & Nutman., 2004) Eosinophils were often the first cell type recruited to the site of infection, and eosinophilia often occur early following infection (Klion & Nutman., 2004). Apart from the rapid influx into the site of infection, eosinophils also exhibited morphological and functional changes that attributed to eosinophil activation. These included changes in cell density, increased surface expression of activation markers, enhanced cellular cytotoxicity and release of granular proteins, cytokines, leukotrienes and other mediators of inflammation (Klion & Nutman, 2004). Basophils have gained prominence recently due to the possible role in Th2 cell differentiation as providers of initial IL-4 and even as APCs. It was understood
that basophils in humans and mice readily generated large quantities of IL-4, in response to various stimuli, including filarial antigens, with or without dependence on IgE (Mitre et al., 2004). In murine filarial infections, effector mechanisms involved multiple innate immune cells, with antibodies acting as initiators of immunity by activating Fc receptor-expressing cells. Basophils have their ability to produce high levels of IL-4, act as effectors to promote filarial killing in secondary or challenge infections (Torrero et al., 2010). Although eosinophils were crucial contributors to the early IL-4 pool, they were also shown to be important in protection against primary filarial infections (Ramalingam et al., 2005), (Simons et al., 2005). The mechanism of protection mediated by eosinophils is thought to antibody-dependent cell-mediated cytotoxicity or through release of eosinophil granule contents.

**Pathogenesis of filarial disease**

The most severe clinical manifestations of lymphatic filariasis were lymphedema and elephantiasis. The first major insight into the role of lymphatic damage in the pathogenesis of lymphatic filarial disease came from Brugian infections on animal studies. As seen in nude mice, pro-inflammatory cytokines of innate origin appeared to play an important role in Brugian infection with the elevated levels of IL-1, IL-6, TNF-α and GM-CSF in lymph fluid (Rao et al., 1996). Hence it was shown in animal studies, the innate cytokines appeared to play a prominent role in the initiation of pathology in filarial-infected. The importance of pro-inflammatory cytokines was also seen in human studies as well in either the early or late stages or lymphedema. Studies have shown that individuals with chronic lymphatic pathology had elevated levels of C-reactive protein (an acute phase protein, indicating an acute inflammatory response), pro-inflammatory cytokines such as
TNF-α, IL-6 and soluble TNF receptor, endothelin-1, IL-2, as well as IL-8, MIP-1α, MIP-1β, MCP-1, TARC and IP-10 in systemic circulation (Babu & Nutman, 2012) Similarly, it was shown that patients with both acute and chronic manifestations of LF had elevated circulating levels of IL-6 and IL-8, also it was shown that those with chronic disease manifestations only had elevated levels of sTNF receptors (Satapathy et al., 2006). Another important mechanism of immune activation in chronic infections was the presence of microbial translocation with elevations in the circulating levels of microbial products. Microbial translocation across the intestine or across the lymphatics could possibly contribute to inflammation and innate immune activation. It was shown that increased circulating levels of LPS, a marker for microbial translocation and decreased levels of LPS-binding protein (LBP) were characteristic features of filarial lymphatic pathology (Anuradha et al., 2012). The same group also demonstrated that microbial translocation was associated with development of an acute phase response with the presence of markers of inflammation in plasma like C - reactive protein (CRP), alpha-2 macroglobulin, serum amyloid protein-A and haptoglobin (Anuradha et al., 2012) Also, increased serum levels of proinflammatory cytokines like IL-1β, IL-12, and TNF-α and IL-6, were associated with progressive immune activation in filarial pathology. Apart from systemic immune activation, progressive fibrosis and extracellular matrix remodeling was another important feature of filarial pathology. Matrix metalloproteinases (MMPs) are proteolytic enzymes that control matrix remodeling and collagen turnover. These MMPs and their inhibitors [tissue inhibitors of metalloproteinases (TIMPs)] were mostly produced by macrophages, granulocytes, epidermal cells and fibroblasts. The dysregulation of MMPs and TIMPs was known to determine the development of pathology in viral, bacterial and
fungal infections, apart from parasitic infections. Recent data suggested that increased circulating levels of MMPs and TIMPs were and the altered ratios of MMP/TIMP were an important underlying factor in the pathogenesis of tissue fibrosis in filarial lymphatic disease. In addition, this was correlated with elevated levels of Type 2 cytokines known to be intimately involved in fibrosis – IL-5, IL-13 and TGFβ (Anuradha et al., 2012).

**Strongyloides infection**

*Strongyloides stercoralis* is an intestinal nematode that infects 3 million to 100 million people worldwide. Although this parasite in most cases causes asymptomatic infection, an alteration in host immune status such as corticosteroids can lead to a fatal, fulminant infection. One reason this disease has been neglected is of the fact that its prevalence has been severely underestimated. Moreover, diagnosis of this disease requires special detection methods which are not available in the routine screening methods that were utilized to identify other parasitic infections in population surveys (Bonne-Annee et al., 2011), (Bethony et al., 2006). Also, the parasites were shed in low and inconsistent numbers that further complicate the diagnosis. It was shown in certain studies that the use of recombinant antigen-based serological diagnosis had greatly expanded the detection rates of this infection within endemic populations (Krolewiecki et al., 2010), (Montes et al., 2010). Although chemotherapy (albendazole or ivermectin) is available for *S. stercoralis* infections, efficacy is rarely 100% (Horton, 2000), (Ikeda, 2003).

**Life cycle of strongyloides infection**

The strongyloides life cycle is known to be more complex than most nematodes with its alternation between free-living and parasitic cycles, and its potential for autoinfection and multiplication within the host.
The two types of cycles that exist are the “Free-living cycle”, where the rhabditiform larvae passed in the stool can either molt twice and become infective filariform larvae which is otherwise known to be called as direct development or molt four times and become free living adult males and females that would eventually mate and produce eggs from which rhabditiform larvae would hatch. The latter in turn could either develop into a new generation of free-living adults, or into infective filariform larvae. The filariform larvae would penetrate the human host skin to initiate the parasitic cycle. The other cycle that exists is the “Parasitic cycle”, where the filariform larvae in contaminated soil would penetrate the human skin, and are transported to the lungs where they penetrate the alveolar spaces; they were then carried through the bronchial tree to the pharynx, were swallowed and then reached the small intestine. In the small intestine they would molt twice and
become adult female worms. The females lived threaded in the epithelium of the small intestine and by parthenogenesis would produce eggs, which eventually would yield rhabditiform larvae. The rhabditiform larvae can either be passed in the stool, or can cause autoinfection. In autoinfection, the rhabditiform larvae would become infective filariform larvae, which could then penetrate either the intestinal mucosa (internal autoinfection) or the skin of the perianal area (external autoinfection); in either case, the filariform larvae may follow the previously described route, being carried successively to the lungs, the bronchial tree, the pharynx, and the small intestine where they would mature into adults; or they may be disseminated widely in the body. To date, occurrence of autoinfection in humans with helminthic infections can be seen only in *Strongyloides stercoralis* and *Capillaria philippinensis* infections. In the case of *Strongyloides*, autoinfection could explain the possibility of persistent infections for many years in persons who have not been in an endemic area and of hyper infections in immunosuppressed individuals (Centers for Disease Control: Global Health – Division of Parasitic Diseases and Malaria, July 2013).

**Clinical manifestations of strongyloides infection**

The clinical manifestations were usually acute or chronic strongyloidiasis. The clinical manifestations of acute strongyloidiasis can be associated with the path of larval migration from the skin to the small intestine. Infected individuals may have experienced irritation at the site of skin penetration by larvae, followed by tracheal irritation or dry cough, and ultimately gastrointestinal symptoms (e.g., diarrhea, constipation, abdominal pain, anorexia) (Keiser & Nutman, 2004). Chronic strongyloidiasis could most frequently cause asymptomatic infection in immunocompetent individuals. Up to 75% of people may
have peripheral eosinophilia or elevated immunoglobulin E levels; therefore, Strongyloides should be considered in the differential diagnosis of high-grade and/or persistent eosinophilia in travelers or expatriates from endemic areas (Nutman, 2007). Symptomatic individuals may have complaints of diarrhea, constipation, intermittent vomiting, or borborygmus (Keiser & Nutman, 2004). Chronic urticaria (Leighton & MacSween, 1990) or larva currens which is associated with pruritic linear streaks located along the lower trunk, thighs, and buttocks may be seen as presenting signs or symptoms of infection. Unusual manifestations of chronic strongyloidiasis include arthritis (Richter et al., 2006), nephrotic syndrome (Hsieh et al., 2006), chronic malabsorption (Atul et al., 2005), duodenal obstruction (Suvarna et al., 2005), (Harish et al., 2005), focal hepatic lesions (Gulbas et al., 2004), and recurrent asthma (Tullis, 1970).

**Diagnosis of strongyloides infection**

In chronically infected, asymptomatic individuals, strongyloidiasis diagnosis has been quite a challenge. Definitive diagnosis relies on detection of larvae in the stool. However, intermittent and scanty excretion of larvae limits the utility of stool studies. Various investigators have attempted to improve the diagnostic yield of stool exams using techniques such as direct smear of feces in saline/Lugol’s iodine stain, Baermann concentration and quantitative formalin ethyl acetate concentration technique (Siddiqui & Berk, 2001). Enzyme linked immunosorbent assay (ELISA) has been increasingly used in conjunction with stool studies to increase diagnostic sensitivity. The high negative predictive value of the ELISA can be particularly useful in excluding strongyloidiasis as part of the differential diagnosis. Various techniques have been developed in an effort to improve on the drawbacks of serologic-based assays. Recombinant antigens (e.g., the NIE antigen) have been proposed as a convenient alternative to the crude antigen currently used.
(Ravi et al., 2002) (Ramanathan et al., 2008). Recently, a dipstick assay was found to be easily and quickly performed and correlated well with ELISA results (Van Doorn et al., 2007).

**Treatment of strongyloides infection**

To prevent the development of chronically infected, asymptomatic individuals must be treated with deworming drugs. Because it has been reported that even a single remaining adult female can multiply and cause disseminated disease, the goal of treatment was complete eradication of the parasite. The current treatment of choice for chronic strongyloidiasis is single-dose ivermectin.

**Immune responses to strongyloides infection**

Humans appear to control infection with *S. stercoralis* through a T-helper type 2 (Th2) response based on the observation that patients co-infected with HTLV-1 and *S. stercoralis* have decreased production of Th2-type cytokines, increased IFN-γ production, and greater susceptibility to hyper infection (Montes et al., 2009), (Neva et al., 1998), (Porto et al., 2001). Th2 responses are the hallmark of many helminth infections with expression of IL-4 and IL-5 essential for control of *Onchocerca volvulus* (Lange et al., 1994), *Heligmosomoides polygyrus* (Urban et al., 1991), *Trichuris muris* (Else et al., 1991), and *Angiostrongylus cantonensis* (Sasaki et al., 1993). Protective adaptive immunity to *S. stercoralis* larvae in mice requires CD4+ but not CD8+ T cells (Rotman et al., 1997). As seen in a study where immunized mice treated with recombinant IL-12 showed a pronounced transfer from a Th2 to a Th1 response and thus blocked mice from developing protective adaptive immunity. In addition, depletion of the Th2-associated cytokines IL-4 or IL-5 from immunized mice using monoclonal antibodies impaired larval killing (Rotman
et al, 1997). The requirement for a Th2 response for protective immunity also has been reported for *S. venezuelensis* (Fernandes et al., 2008) and *S. ratti* (Bleay et al., 2007). *S. venezuelensis* infections in Lewis rats shifted the immune response from Th1 during acute infections to Th2 during the recovery phase (Chiuso-Minicucci et al, 2010) and IL-12-deficient mice infected with *S. venezuelensis* had higher levels of Th2 cytokines and decreased parasite burdens (Machado et al., 2009). Primary infections of rats with *S. ratti* induced a Th2 response within 2–3 weeks post infection (Wilkes et al., 2007), which resulted in the production of IL-4, IL-5, and IL-13 and a suppression of IFN-γ in mice and rats (Eschbach et al., 2010), (Paterson et al., 2008).

The frequent absence of eosinophils associated with migrating larvae suggested that while peripheral eosinophilia was a common systemic response, eosinophils may not primarily be involved in the tissue response to the parasite. Lymphocytes, macrophages, and neutrophils were known to be frequently seen in close association with the larvae in various tissues; however, their role in controlling the infection has not been elucidated (Genta et al., 1989), (Haque et al., 1994). Also, the data on human protective immune responses to *S. stercoralis* were limited, and analysis of the data has been restricted to identifying correlations between immune responses and disease states only. Hence, *in vitro* and animal models were required to define the actual role of cells in immunity to the *S. stercoralis*. Studies have shown that, depleting IL-5 from mice immunized against infection with *S. stercoralis*, either by monoclonal antibody treatment (Rotman et al., 1996), (Rotman et al., 1997) or by genetically knocking out IL-5 (Herbert et al., 2000), resulted in decreased numbers of eosinophils and a loss in protective adaptive immunity. But, when eosinophils were specifically absent, either due to elimination by monoclonal antibody treatment
(Galioto et al., 2006) or the use of PHIL mice that were genetically deficient in eosinophils (O’Connell et al., 2011), it was demonstrated that eosinophils were not required as effector cells in the adaptive immune response.

Interestingly, immunized IL-5-deficient mice, which had severely reduced numbers of eosinophils, failed to establish protective immunity and had lower levels of parasite-specific IgM (Herbert et al., 2000). Reconstitution of immunized IL-5-deficient mice with wild-type eosinophils elevated the parasite-specific IgM levels, and the mice were then able to eliminate infection challenge (Herbert et al., 2000). Similarly, it has been reported that IgM induced by the adjuvant alum is compromised in mice genetically deficient in eosinophils and that transfer of IL-4 expressing eosinophils restored the production of antigen-specific IgM (Wang et al., 2008), therefore confirming a role for eosinophils in IgM production. Immunized PHIL mice, which had no eosinophils but intact cytokine levels, did not have reduced IgM levels (O’Connell et al., 2011). The immunized PHIL mice appear to have an alternative source for molecules required for the induction of IgM production that IL-5\(^{-/-}\) mice did not have. Therefore, eosinophils function as effector cells in the innate immune response, antigen-presenting cells and as sources of cytokines required for IgM production in the adaptive immune response.

**Tuberculosis (TB) infection**

*M. tuberculosis (Mt)*b is the main causative agent of human TB and kills nearly 2 million people every year with its highest prevalence in the developing countries. It has been reported time and again that an estimated 2 billion people worldwide are latently infected with nine to ten million new cases every year (Parida and Kaufmann, 2010).
financing: WHO report 2009). It has also been estimated that the global burden of TB remains enormous with an estimated 8.7 million new cases of TB and 1.4 million deaths due to TB in 2011 (World Health Organization: Global tuberculosis control, WHO report 2012). The only vaccine available yet is the Bacille-Calmette-Guérin (BCG), although it is well known that it does not protect adults against pulmonary TB and only few discoveries in the field of anti-TB drugs have taken place in the last 40 years. It has to be noted that the genus Mycobacterium originated more than 150 million years ago (Hayman, 1984), since then the pathogen has been growing slowly with a doubling time of up to 48 hours. It has been reportedly classified as an acid-fast bacterium, because of its ability to be stained by only certain dyes, and is coated with a thick and unique cell wall that is made up of mycolic acids which are connected via arabinogalactan polysaccharide to the conventional peptidoglycan layer (Brennan, 2003).

**Clinical manifestation of TB infection**

It has been well documented that, infection with *Mtb* generally occurred by the inhalation of droplets containing the pathogen generally through close contact with a patient with active pulmonary TB. In most cases this could lead to an infection in the lung, but could also affect other organs in the body. It has been shown that the bacteria which reside in the alveolar space would encounter alveolar macrophages and DCs and eventually gets engulfed (Velasco-Velazquez et al., 2003), (Ferguson et al., 2004). Once internalized, *Mtb* gets encapsulated in the phagosome and would block phagosome acidification and actively interfere with phagolysosomal fusion by immune evasion mechanisms (Armstrong & Hart, 1971). In addition, the bacteria were also able to persist and proliferate within the phagosome and the infected cells transport the bacteria into the
lungs. The production of TNFα and inflammatory chemokines from the infected macrophages would then drive the recruitment of neutrophils, NK cells, CD4+ and CD8+ T cells, each of which would produce their own set of chemokines and cytokines that lead to the remodeling of the infection site into a structure called the granuloma (Russell, 2007), (Ulrichs & Kaufmann, 2006), (Algoo et al., 2003). The formation of a stable granuloma is known to be responsible for the immune containment during the latent, or subclinical, period of the infection.

**Figure V: Infection cycle of M. tuberculosis.** Adapted from Kaufmann, S. H. and McMichael, A. J., Nature Medicine, “Annulling a dangerous liaison: vaccination strategies against AIDS and tuberculosis”, 2005.

It has been reported that, the vast majority of Mtb infected individuals will not succumb to active TB but would remain latently infected for the duration of their lives (Figure V). Reactivation would only occur in 5 to 10% of infected people and could be triggered by
various factors such as immunosuppression due to age, corticosteroids, malnutrition and others (Flynn, 2004). It also has to be noted that most importantly co-infection with HIV would increase the risk of developing active pulmonary TB by several folds (Kaufmann & McMichael, 2005). Also a striking feature of *Mtb* was that the ability to survive under hypoxic and nutrient-poor conditions by shifting to a dormant life stage. This was characterized by alternative energy catabolism and a thickening of the cell wall by the activation of the transcription factor, Rv3133c that would eventually activate 48 differentially regulated genes have been identified for dormancy (Cole et al., 1998), (Graham & Clark-Curtiss, 1999), (Cunningham & Spreadbury, 1998), (Wayne, 1994), (Sherman et al., 2001). Deletion of this dormancy survival regulator (DosR) led to the loss of persistence in hypoxic conditions (Park et al., 2003), (Boon & Dick, 2002). Apart from dormancy, resuscitation promoting factors (Rpf) have been shown to be associated with the reactivation of the bacteria from a dormant stage to active replication (Biketov et al., 2000), (Cohen-Gonsaud et al., 2005). The very mechanisms facilitating resuscitation of *Mtb* and subsequent disease reactivation still remains poorly understood.

**Diagnosis of TB infection**

For over 100 years, the standard diagnostic test for *M. tuberculosis* infection has been the tuberculin skin test (TST) which is purified protein derivative (PPD) antigens from metabolically active *M. tuberculosis*. Currently, PPD is being used intra-dermally in the Mantoux technique to diagnose TB. It has been reported that a negative TST does not necessarily exclude infection with *M. tuberculosis*, since it was demonstrated that up to 25% of people with active TB are nonreactive to the Mantoux test. Another disadvantage of the TST is the compromised specificity due to numerous shared antigens with the BCG
vaccine and non-tuberculous mycobacteria (NTM). Further studies have shown that, in addition to the TST, microscopic examination of acid fast-stained sputum, with Ziehl-Neelsen (ZN) was one of the easiest, most inexpensive and rapid methods used in most developing countries. There were also reports suggesting that the culture method is even more sensitive than microscopy and is used additionally in most resource-limited countries (Yeager et al., 1967). Later studies have shown that a modification of this technique is the liquid media support growth that had an increased recovery of positive cultures. Furthermore, studies have shown that chest radiography could identify calcified lesions in the parenchyma of the lungs that could indicate a granuloma. Some studies have demonstrated that IFNγ release assays (IGRA) were a new set of diagnostics using *M. tuberculosis*-specific antigens that reduce the risk of false positivity due to cross-reaction with other mycobacterial strains. Of late, the two IGRA formats that have been officially approved in many countries were the Quantiferon – TB Gold® and the T-Spot-TB® diagnostic tests. Both systems relied on host reaction to infection that measured IFNγ produced by T-cell responses to the *M. tuberculosis*-specific antigens 6kDa early secreted antigenic target (ESAT6) and 10kDa culture filtrate protein (CFP-10). These antigens were found to be encoded by the region of difference-1 (RD-1), which could be seen in the genome of *M. tuberculosis* but was not present in BCG and most other mycobacteria (Lighter & Rigaud, 2009). While the Quantiferon – TB Gold® is based on an IFNγ enzyme-linked immunosorbent assay (ELISA), the T-Spot-TB® employs an *ex vivo* enzyme-linked immunospot assay.
Immune responses to TB infection

It is understood that both immunity and pathogenesis were mediated by the lymphocyte response to mycobacterial infection. Therefore, the absence of an acquired cellular immune response lead to limited or no immunity, also it limited the classical caseation associated with pathogen transmission. The most important aspect of the acquired cellular response was the rapidity with which it is expressed. If the response was too slow, bacteria would grow to a point where although a protective response was being expressed, the environment was ineffective. Along the same lines, it was clear that dosage plays a role in the ability of the host to control bacteria. Specifically, if one was infected with high dose, the local bacterial burden would have reached a level that interferes with the efficient expression of protective immunity. These concepts were demonstrated by Rich (Rich, 1944) using the lung histopathology from patients in the pre-drug era to describe the natural history of the disease. In his study, he had suggested that the acquired cellular response could control bacterial growth but it failed to do so when the bacterial numbers were too high.

Various studies have shown that DCs were considered to be the most efficient inducers of activation in naive T cells. This efficiency was drawn from the fact that they not only provide the antigen-specific stimulus but also other signals that promote efficient development of effector T cells. The effect of mycobacterial infection on DC function has been studied extensively. Indeed, the classic demonstration that immature DCs could perform phagocytosis and become efficient APCs by using BCG as the maturation agent (Montminy et al., 2006). However, the ability of Mtb to interfere with T cell stimulation has been suggested by the fact that DCs infected in vivo were less efficient at stimulating
antigen-specific T cells than the equivalent uninfected DCs (Robinson et al., 2008). It was shown that, once bacteria reached the draining lymph node, initiation of naive T cell activation would occur and discussed that this phenomenon was a result of direct interaction between lung-derived bacteria-infected DCs or not has not been definitively proven. Furthermore, studies have shown that IL-12p40 promotes this migration, whereas IL-10 may limit it (Wolf et al., 2007), (Khader et al., 2006), (Nigg et al., 2007). These data suggested that simple exposure to Mtb was not sufficient to limit the ability of an infected DC to initiate T cell activation. Infected lung DCs migrate from the lung to the draining lymph node, but this migration was difficult to see before day 14 of infection, and this was after the initial migration and initiation of T cell activation (Robinson et al., 2008). That this activation occurs with the expected kinetics indicated that the simple presence of the bacteria in the lymph node does not affect initial T cell activation.
Figure VI: Low-dose aerosol infection, which approximates the natural delivery route for induction of tuberculosis, results in low numbers of Mtb (red) being deposited in the lower airways and the alveolar tissue. Bacteria do not disseminate from the lung until 9 days post infection, when they can be detected in the draining lymph nodes. This dissemination coincides with the first activation of naive T cells (purple). The fact that bacteria do not disseminate rapidly suggests that they either inhibit migration of dendritic cells or that the cells that they infect cannot migrate readily to the lymph node. Activation of naive T cells occurs in the presence of live bacteria, and effector cells develop with expected kinetics. The effector cell phenotype will depend on the availability of specific cytokines. These effector cells migrate to the lung in response to inflammation and mediate protection by activating infected phagocytes (pale red). The response takes 18–20 days to reach an effective level and thereby to stop bacterial growth. Adapted from Cooper AM, 2009, Annu. Rev. Immunol.
Once cells were activated, they must migrate to the primary site of infection, and this migration takes place 15–18 days post infection (Reiley et al., 2008) (Figure VI). Once T cells were activated, they expand and were recruited to the infected lung where they are thought to mediate control of bacterial growth through IFN-γ-induced activation of infected phagocytes. This pathway of control was based on the data from one of the studies that showed the primary correlate of bacterial control was the expression of IFN-γ mRNA in the lung and that antigen-specific CD4+ T cells were capable of making IFN-γ arrive at the same time as the cessation of bacterial growth in the lung. This accumulation of IFN-γ-producing CD4+ T cells in the lung was dependent upon IL-12p70, and in its absence, the numbers of such cells were reduced (Cooper et al., 2002). Despite this low number of cells, reduced growth of bacteria does occur in the absence of IL-12p70 (as seen in the IL-12p35 gene-deficient mice), but this response cannot be maintained for a prolonged period of time. The same group had demonstrated that in the absence of IL-12p35, the resulting antigen-specific IFN-γ-producing cells were dependent upon IL-23, as IL-12p35 IL-23p19 double gene-deficient mice were as unable as IL-12p40-deficient mice to generate IFN-γ-producing cells capable of limiting bacterial growth within the lung (Khader et al., 2005). However, the same group demonstrated that in the absence of IL-12p35, there was an enhanced antigen-specific IL-17-producing population, which may reflect a regulatory role for either IL-12p70 or IL-35 in this response (Khader et al., 2005).

Indeed, although it is widely assumed that CD4+ T cells making IFN-γ were required for protective immunity, this assumption was based largely on correlative data. In particular, it was clear that cessation of bacterial growth correlates with arrival of IFN-γ-producing CD4 T cells (Orme et al., 1992), (Chackerian et al., 2001), (Jung 2005), (Khader et al., 2007) and
that loss of CD4⁺T cells increased the likelihood of succumbing to tuberculosis (Havlir & Barnes, 1999). Clearly, various CD4⁺T cell effector subtypes have been shown to exist, ranging from early activated cells that make only IL-2, to cells making IFN-γ, to multifunctional cells expressing IL-2, IFN, and TNF, and the presence of these multifunctional cells was associated with protection (Darrah et al., 2007). Furthermore, cytolytic CD4⁺T cells have been identified in mice (Orme et al., 1992) and humans as well (Klucar et al., 2008). Multifunctional cells were seen at high frequencies in tuberculosis patients (Winkler et al., 2005) and also in those people in highly endemic areas (Scriba et al., 2008) and in vaccinated infants (Soares et al., 2008). Development and function of all effector cells were depended on the ability of DCs which were present in the draining lymph node so that they would prime and promote survival of these cells efficiently. This priming required expression of antigen in the context of MHC, co-stimulatory molecules, and the necessary cytokines that promoted T cell polarization. However, the conditions required to generate and maintain the multifunctional and cytolytic antigen-specific lymphocytes have not been fully defined (Lindestam., 2014).

In another study, they had demonstrated the role of IL-17 in the comparison between Mycobacterium bovis BCG and Mtb infection, as the antigen-specific IL-17 response was down regulated by IFN-γ during BCG infection (Cruz et al., 2006) whereas this regulation was not as strong during Mtb infection when both an IFN-g and an IL-17 antigen-specific response were detected throughout infection (Khader et al., 2005). They had stated that it may be due to BCG and Mtb activate DCs to slightly different degrees and that this was reflected in the relative ability of each bacterium to drive specific effector cells. The group also measured cytokine production by DCs infected with M. bovis BCG, and found that
while IL-23 was induced by DCs, IL-12p70 was produced only when IFN-γ was present (Cruz et al., 2006). Also the level of other cytokines present during activation of naive T cells is instrumental in the differentiation process, especially transforming growth factor-β (TGF-β) or IL-6, as these cytokines were crucial to the development of IL-17-producing T cells, and it was their relative levels which differentiated between regulatory and Th17 cells (Ivanov et al., 2007), (Zhou et al., 2008).

Investigators had recently identified other subsets of functional T cells, such as those producing IL-17 and IL-22, and these cells have been seen in the mouse model and in humans exposed to tuberculosis (Khader & Cooper, 2008). Although their protective in tuberculosis has been questionable; however, their recent discovery demonstrated that when IL-17-producing antigen-specific cells were induced in mice following aerosol infection showed that these cells and indeed most of the IL-17 response in the lung depended on the presence of IL-23 (Khader et al., 2005). It was also noted that γδ T cells were a source of IL-17 and produced this cytokine very early after a high-dose intranasal challenge with BCG (Umemura et al., 2006). It was shown that a large portion of the IL-17 response in the mouse model was within the γδ T cell population (Lockhart et al., 2006). They had also showed that when IL-17 was blocked during a high-dose challenge, neutrophil recruitment was hindered, and this may alter subsequent development of inflammation (Umemura et al., 2006). Whether these cells were protective or damaging is still unclear, but when Mtb-infected animals were repeatedly challenged with mycobacterial antigen, the lesions became necrotic and contain a higher frequency of granulocytes (Taylor et al., 2003), (Turner et al., 2000). These data suggested that the nature of the inflammation that developed in response to chronic antigen exposure depended on IL-23 and IL-17. In
addition, it was also understood that there was the potential for a dual role for these two cytokines in the response to Mtb infection in the mouse. Whether this response was equally important in humans is as yet unknown; however, CD4+ antigen-specific IL-17- and IL-22-producing cells could clearly be detected in humans exposed to Mtb, although only IL-22 has been shown to be detected in the lung (Scriba et al., 2008).

A second function important in anti-mycobacterial activity is autophagy. Cells perform autophagy by sequestering their own cytoplasm into an autophagosome that is then delivered to the lysosome (Levine & Klionsky, 2004). IFN-γ induces autophagy, and inhibition of autophagy increased the viability of intracellular mycobacteria in mice and humans alike (Gutierrez et al., 2004), (Singh et al., 2006). Interestingly, this activity has been linked to immunity-related p47 guanosine triphosphatases, one of which, Lrgm1 (LRG-47), was essential for IFN-γ-mediated control of mycobacterial growth in mice (MacMicking et al., 2003); the human ortholog of this gene, IRGM, also has been shown to play a role in autophagy and the control of mycobacterial burden (Singh et al., 2006). It was shown in a study that the autophagic response to Mtb was abrogated by IL-4 and IL-13 and enhanced by TLR4 ligation (Xu et al., 2007).

With regard to induction of the acquired response, it was reported that, on the basis of specific TLR expression, both DCs and macrophages had different responses to Mtb (Bafica et al., 2005), (Pompei et al., 2007). The level and activity of TLR-mediated signaling by DCs encountering the bacteria would influence the outcome of the cellular response, but in low-dose aerosol infection any differences may result in subtle outcomes early in infection and may only be seen by careful analysis. For example, the relative levels of IFN-γ- and IL-17-producing cells during mycobacterial infection in both mouse and
human would depend on the level of specific cytokines present during and after T cell activation (O'Garra et al., 2008). A recent analysis of the response of human DCs to Mtb found that IL-23 was preferentially expressed, likely in a TLR2-dependent manner, compared with IL-12p70 in the absence of IFN-γ, apart from these cytokines; it was also shown that IL-10 was also induced. It was demonstrated that following IFN-γ activation, DCs responded to Mtb by producing both IL-23 and IL-12p70 and by reducing the level of IL-10 (Gerosa et al., 2008). These data suggested that in humans, before expression of IFN-γ, the preferential T cell effector type would have likely been an IL-17 producer but that thereafter both the IFN-γ and IL-17 response would be promoted. But in the mouse model, it was shown that Mtb-infected DCs generated IL-12 and IL-23 and promote both IFN-γ and IL-17 populations in CD4 T cells (Khader et al., 2005).

**Helminth-TB Co-infections**

It has been estimated that one-third of the world's population harbors any one of the various species of intestinal parasite (Hotez et al., 2011), (Crompton, 1999), (de Silva et al., 2003). Coincidentally, helminthic infections has shared the same ‘developing world niche’ as does tuberculosis disease. These parasitic helminths are a highly diverse, multicellular species that appear to have evolved to cause a variety of diseases by finding definitive dwelling places in different organs of the host. Various studies have demonstrated their complex life cycle that involves a migratory pattern from an intermediate to a definitive host, wherein the different growth stages of the parasite, after gaining entry either through penetration through the skin or by the oral route could spend their entire life cycle in the gastrointestinal tract or traverse through the lungs, liver and brain to establish chronicity. Co-infection with multiple helminthes could be a stochastic event with the probability of
multiple infections increasing in areas of endemicity. Indeed, co-infections do occur quite frequently in humans with symbiotic and/or competitive outcomes, and therefore it has been argued that helminth-triggered Th2 cytokines can affect protective immune response to mycobacteria (Bentwich et al., 1999). In one study, it was shown helminth-induced skewing of anti-Mtb Th1 effector mechanisms and highlighted those helminth-induced alternatively activated macrophages (M2) down modulated local pulmonary anti-Mtb defense mechanisms. (Rafi et al., 2012).

Several studies have demonstrated that helminths induce a strong Th2 response in the host that promotes mucus secretion, collagen deposition and wound healing mechanisms that were critical for helminth expulsion (Bentwich et al., 1999). Despite induction of the protective Th2 response, helminths were often able to persist in the host for a long time, resulting in chronic infection (Allen & Maizels, 2011). Persistence in the host is achieved, in part, by the induction of immunoregulatory pathways, as observed that during helminth infection, expanding populations of Tregs produced TGF-β and IL-10 (Babu et al., 2006), (Ince et al., 2006). In humans, reactivation of TB is associated with increased production of IL-10 and TGF-β by circulating monocytes and possibly Tregs, which suppress production of Th1 cytokines (Hirsch et al., 1997), (Ribeiro-Rodrigues et al., 2006). In co infected hosts, helminth-induced generalized anti-inflammatory response would seem to prevent the development of a Th1 response which is critical for host resistance against Mtb infection (Flynn et al., 2011). In contrast, pathogenic microorganisms typically triggered a type 1 immune response, which instead results in elevations in IL-12, IL-23, IFN-γ and IL-17 (Babu et al., 2006). This potent response developed rapidly, which is critical for the control
of potentially lethal pathogens that can rapidly divide and disseminate throughout the host; however, a cost of this rapid response can be tissue-damaging inflammation. Major global pathogens, including *Mycobacterium tuberculosis*, human immunodeficiency virus (HIV) and the *Plasmodium* species that cause malaria, have an overlapping geographical distribution with helminth infection (Figure VII). The overlapping geographical distribution emphasizes the point that in South East Asia there is extensive overlap of the presence of helminths, malaria, and *M. tuberculosis*. Widespread concurrent infections with helminths and other pathogens may pose a clinical and immunological uncertainty, as co-infected hosts must manage two classes of pathogens that generate disparate and potentially conflicting effector-cell responses for their control and resolution. Hence, it is understandable that in most part of the world, host immune responses to these prominent disease-causing microbial pathogens can be modulated by co-infection with helminths, and may also potentially impair vaccine efficacy (Sabin et al., 1996), (Elliott et al., 2010). The chronicity of helminth infections from childhood through adult life may be affecting the overall immunological function. Studies have suggested that helminth infection may influence homeostasis of the immune system, activating immunoregulatory cell populations and Th2 effector cells that together control the development of harmful autoimmune and inflammatory disorders. Consistent with that model, populations in regions without endemic helminth infection show an increased prevalence of inflammatory diseases and autoimmunity (Elliott & Weinstock, 2012). Studies of experimental models have further demonstrated the efficacy of helminth infection or the administration of helminth products in mitigating disease severity (Elliott et al., 2004), (Zaccone et al., 2009), (Mishra et al, 2013), which has provided the basis for many ongoing clinical trials.
A study on filarial-TB co-infections demonstrated that, in-vitro responses to non parasitic antigens including the mycobacteria antigens (purified protein derivative (PPD), culture filtrate protein (CFP), and crude Mtb extract] appear to remain largely intact (as measured by lymphocyte proliferation and/or IFN-γ production) in those with filarial infection, and Type-1 responses to mycobacteria antigens are relatively normal (Babu & Nutman, 2011). Another study reported that the low proliferative response and IFN-γ production of PBMC to PPD stimulation is enhanced following antihelminthic treatment (Elias et al., 2001). Lymphocytes from mice co infected with M. avium and Schistosoma mansoni were found to be blunted in their production of IFN-γ in response to PPD stimulation (Sacco et al., 2002). Additionally, PPD-specific Th1 responses were found to be skewed by presence a priori of a Th2-inducing parasite in mice (Pearlman et al., 1993). Notably, concomitant
intestinal helminth infection in tuberculosis patients was found to negatively affect the T-lymphocytic population, which also was associated with lower levels of IFN-γ (Resende et al., 2007). A similar decrease in IFN-γ was also seen in lepromatous leprosy patients coinfected with helminthes (Diniz et al., 2010). Furthermore, a concurrent filarial infection was found to inhibit the generation of potentially protective Th1 immune responses in patients with LTBI (Babu et al., 2009). One study also demonstrated that PPD-specific and CFP-specific Th1 and Th17 responses are significantly lower in latent TB-infected individuals with lymphatic filariasis compared with those without filarial infections and this down modulation is not mediated through IL-10 or TGF-β but through the increased expression of the negative co stimulatory molecules – CTLA-4 (cytotoxic T-lymphocyte antigen 4) and PD-1 (programmed cell death 1). Hence, filarial infections have been shown to have a major immunological impact on the mycobacterial antigen-specific immune responses in latent tuberculosis (Babu et al., 2009).

An important issue for TB control is the broad variability of BCG vaccination efficacy reported worldwide. It has been understood that a likely cause of such variability is due to the presence of intestinal helminth infection and consequent impairment of immune response to recall antigens (Elias et al., 2005), (Elias et al., 2006). A study had shown that following BCG vaccination in infants sensitized in utero to helminthes demonstrated a PPD-induced IFN-γ production significantly lower relative to those infants who were not prenatally sensitized (Malhotra et al., 1999). Further, understanding the idea that chronic helminthic infections may affect the protection of BCG in children. Elias et al also reported that antihelminthic treatment prior to BCG vaccination in adults lead to increased frequency of IFN-γ and IL-12 producing cells in PPD-stimulated PBMC cultures 2 months
after vaccination in comparison with the placebo group that did not receive anti-helminth treatment.

Research over the last decade had identified a multitude of mechanisms for diminished MTB-antigen-stimulated production of IFN-γ at the time of diagnosis of active TB (Hirsch, et al., 1999), (Toossi et al., 1986), (Hirsch et al., 1996), (Gong et al., 1996), (Hirsch et al., 1997), (Hirsch et al., 1996). A study showed that, 27.5% (11 of 40) of all patients with TB, were positive for at least one intestinal helminth, confirming previous findings (Tristao-Sa et al., 2002), (Elias et al., 2006). Interestingly, infection with S. stercoralis was found in 72.7% (8 of 11) of TB + Helm patients. Infection with S. stercoralis has been associated with a strong Th2-type immune response (Carvalho & Da Fonseca Porto, 2004). However, mechanisms responsible for the prolonged delay in recovery of MTB-specific T cell IFN-γ production by peripheral blood mononuclear cells (PBMC) of TB patients (Hirsch et al., 1999) remain unclear.

Previous studies indicated that active pulmonary TB is associated with a reduction in absolute numbers of both circulating CD4+ and CD8+ T cells (Ribeiro-Rodrigues et al., 2006). Also, lymphopenia had been described in patients with chronic intestinal helminth infection (Onyemelukwe & Musa et al., 2001). Therefore, it was possible that the significant decline in lymphocyte counts in co-infected patients may have been the result of combined effects of both TB and intestinal helminthiasis on the host immune system. Despite eosinophilia was being associated mainly with helminth infections, absolute counts of eosinophils were elevated and comparable in both TB and co-infected patients. The same group had explained that the finding could be TB patients harboured occult helminth infection, despite the fact that all control subjects and patients underwent extensive
examination for helminths in at least three consecutive stool samples investigated by the Lutz (Lutz et al., 1919), Kato-Katz (Katz et al., 1972) and Baerman-Moraes (Baermann et al., 1917), (Moraes, 1948) methods. However, in a previous study from the same group had demonstrated that up to 25% of TB patients without intestinal helminths had eosinophil counts higher than 600 cells/mm³ (Tristao-Sa et al., 2002). They explained that, IL-5 which was an important cytokine in helminth-associated eosinophilia was undetectable in WB culture supernatants from both TB and co-infected patients.

The same group went on to demonstrate that, numbers of Tregs were lower in TB + Helm as opposed to TB patients. One possible explanation for decreased Treg counts in peripheral blood of TB + Helm patients was due to the fact that these cells were sequestered at sites of both active helminth infection in the gut and the lung during active pulmonary TB. In addition, they had also mentioned that it was possible that Treg frequencies in these patients were lower due to the prolonged pulmonary disease observed during TB + Helm infection, which would have sequestered more cells in the lung. In addition, Campanelli et al (Campanelli et al., 2006) showed that functional Treg cells accumulate in skin lesions from patients with cutaneous leishmaniasis. Thus, taken together, they had clearly stated that it was possible that in TB + Helm patients Treg numbers were reduced due to sequestration of CD4⁺CD25<sup>high</sup> T cells at more sites of active helminth and mycobacterial infections, such as gut and lung, respectively.

All these studies have demonstrated that there is a strong plausibility that helminth infections could negatively impact TB disease. The TB field is in a propitious situation to conduct definitive mechanistic studies in humans and understand whether helminth
infections could increase TB incidence and transmission and blunt the protective immune response to vaccines and whether these effects can be reversed anti-helminthic treatment.