Introduction
Introduction

Parasitic diseases have been responsible for major health problems in developing and underdeveloped countries of tropical and subtropical belts. Helminth parasitic diseases, viz., Filariasis, Ascariasis and Hookworm infections contribute significantly to the problem. Human Lymphatic filariasis (LF) is a mosquito borne disease of tropics and subtropics (Sasa, 1976). It is one of the oldest and most debilitating diseases in the world [1], (Dean, 2001). Lymphatic filariasis is caused by infection with the nematode worms *Wuchereria bancrofti, Brugia malayi*, and *Brugia timori*, which are transmitted by mosquitoes. Lymphatic filariasis is a global health problem. At present the World Health Organization has estimated that over 1.25 billion people are at risk in around 72 countries. It is estimated that approximately 120 million people are infected with lymphatic filariasis and over 40 million have pathologic consequences including the disfiguring elephantiasis. *W.bancrofti* accounts for 90% of lymphatic filariasis infections worldwide; while *B. malayi* is prevalent only in some parts of South & South East Asia, *B. timori* is found common only in Indonesia (WHO, 2002).

Globally today, LF remains the second leading cause of permanent and long-term disability after mental illness [2,3]. As a clinical disease it is manifested primarily as acute adenolymphangitis; and chronic lymphedema that may lead to elephantiasis in men and women and to the formation of hydroceles (for *W. bancrofti* at least) in men. One third of the people infected with LF live in India, a third live in Africa and the remainders live in the Americas, the pacific Islands, Papua New Guinea and South East Asia [4].

Th9 cells are a subset of CD4+ T cells, shown to be important in allergy, autoimmunity and anti-tumor responses. However, their role in human infectious diseases has not been explored in detail. These Th9 cells are characterized by the coincident production of IL-9 and IL-10 and develop from naïve CD4+ T cells under the combined influence of IL-4 and TGFβ. The role of IL-9 in human parasitic infections is not known. Th9 cells have been associated with the development of pathology during allergic inflammation and autoimmune disease [48, 49]. It is still unclear whether IL-9 mediates pro-inflammatory or anti-inflammatory activity. Because filarial infection exhibits differences in clinical manifestations with both an inflammatory component (filarial disease) and a non-inflammatory component (asymptomatic infection).

MMPs are a family of zinc-metalloendopeptidases responsible for the turnover of Extra Cellular Matrix Interestingly; among the MMPs with enhanced baseline expression in filarial lymphedema are the two major collagenases in the MMP family, MMP-1 and MMP-8. While collagenases are known to degrade collagen, MMP-9 is also a gelatinase that can degrade collagen as well as gelatin [94]. Lymphatic dysfunction and localized/systemic immunologic and inflammatory responses are important features of lymphatic pathology [82] Although the importance of tissue fibrosis in the pathology associated with LF is well known [24], the molecular mechanisms underlying the fibrotic process in filariasis has not been well established.
Review of Literature
Review of Literature

Historical Perspective

Filariasis is one of the oldest diseases and its history is as old as the history of human civilization itself. The dramatic symptoms caused by the infection of *W. bancrofti* especially the enormous swelling of the legs or scrotum were recorded in the 18th century medical literature of India, Persia, China, and Japan. The mummified body of Natsef-Amun, a priest at Karnak in the time of Rameses XI during 1113-1085 B.C., was proven by autopsy to have lymphatic filarial worms in the groin even after 3,000 years and is still preserved in Leeds museum (Dean, 2001). Historical records of this disease seen in 7-8 A.D. is given as an illustration in 'Yamai zoshi' of elephantiasis leg in a woman which is preserved in the Tokyo National museum [5]. People with elephantiasis were excluded from the Buddhist priesthood during 600-250 B.C.[6]. The Operational Manual on National Filaria Control Programme, published in 1995 by the National Malaria Eradication Programme (NMEP), India reports that Susruta, a physician, mentioned about the disease as 'Sleepad' as early as 600 B.C. Later in A.D. 70, Madhavakara, a pathologist, in his book "Madhava Nidan" described the signs and symptoms of this disease; these signs and symptoms do stand true even today. NMEP (1995) mentions a description of elephantoid legs in Cochin as 'Malabar Legs'. During 16th century Kerala elephantiasis of the leg was attributed to the curse of Saint Thomas as portrayed by Linschoten. All these inferences confirm certainty of *B. malayi* infection in ancient and medieval India [7].
In 1877 Partick Manson first identified the blood-sucking mosquito as the key agent in the transmission of lymphatic filariasis; he is internationally recognized as the father of Tropical medicine. He was the first to identify the non-periodic form of filariasis. However, microfilariae were first described from hydrocele fluid by Demarquay 1863 in Paris and in India from blood by Lewis in the year 1872. Manson in the year 1896 examined the blood films collected in 1884 by Davis in Samoa, who had also reported mf from the blood of persons in Polynesia. Subsequently, he described the localization of mf in lungs and cardiac muscles during the daytime. Manson was acquainted with the periodic type of filariasis, so he assumed that the mf from Samoa to be nocturnal type. Thorpe 1896 observed that the mf from South Pacific were present in the daytime as well as at night and established the existence of non-periodic form of filariasis, which is known to be prevalent in the other islands of the region viz. Fiji, Caledonia, the Loyalty Islands and Polynesia. O'Connor 1923 showed that this form of filariasis was endemic to the islands of Western Pacific. The age specific analysis showed that mf and disease rate in persons aged above 16 years was comparatively more than those under 16 years. The clinical-epidemiological profile of this form of filariasis in this region showed that episodes of filarial fevers associated with lymphangitis and lymphadenitis were the commonly reported signs. Elephantiasis associated with hydrocele was known to manifest later in life [8]. In 1868, Wucherer described a species of worm in the urine of a patient with tropical haematuria in Brazil and clearly distinguished it from Bilhania haematobium. In 1870 he noted that the chylous urine invariably contained nematode worm. After this, Manson found the same worm in the blood of a patient (1899). Subsequently in 1872 Lewis discovered a dead mf in the peripheral blood and four live female worms. Cobbold proposed the scientific name "Filarial bancrofti" and Da Silva araujo proposed the
generic name Wuchereria in 1877. Later, Manson (1879) found the two characteristics of filarial parasite: the transmission by mosquito; and the nocturnal periodicity.

After the World War II, remarkable contributions were made to the epidemiology and control of filariasis. Hewitt et al. discovered the filaricide- diethylcarbamazine (DEC) in 1947 that became the milestone in the history of the anti-filariasis campaign. In 1957 Kessel generated the first set of the result in the control of filariasis by mass administration of DEC (Sasa, 1976). It was Manson (1877), who found mf in the blood of the patients with elephantiasis and associated this and other clinical signs of lymphatic obstruction with the infection (Manson, 1878), also showed that the development of the parasite in the female mosquito after the uptake of mf during its blood meal but wrongly concluded on the mode of transmission of these larvae to man. He postulated that when infected mosquitoes died in water, the filarial larvae were released and the swallowing of these larvae infected man. (Low G.C.1900), on the basis of histological studies on infected mosquitoes, was the first to suggest that human infection took place during the blood meal of an infective mosquito.

The first definitive reports of lymphatic filariasis only began to appear in the 16th century. Lymphatic filariasis known as “the curse of St. Thomas”[7]. was recorded by the Dutch explorer Jan Huygen Linschoten during a visit to Goa between 1588 and 1592 that the descendants of those that killed St. Thomas were “all born with one of their legs and one foot from the knee downwards as thick as an elephants leg” (Burnell A. C. 1885). Another pathological condition associated with lymphatic filariasis is chyluria in which the urine appears milky. William Prout recorded this condition in his 1849 book On the Nature and Treatment of Stomach and Renal Diseases (Prout W. T. 1849). The adult worm was described
by Joseph Bancroft in 1876 [9] and named Filaria bancrofti in his honour by the British helminthologist Thomas Spencer Cobbold (Cobbold T. S. 1877).

Greek and Roman writers were aware of the differential diagnosis of the condition and used the term “elephantiasis graecorum” to describe leprosy and the term “elephantiasis arabum” to describe lymphatic filariasis; the Arabic physicians, including Avicenna, were also aware of the differences between leprosy and lymphatic filariasis (Kiple, K. et al., 1993). Microfilaria in the Dutch East Indies (now Indonesia) which was morphologically different from W. bancrofti was called as Filaria malayi (Brug, 1927; Lichtenstein, 1927; Brug, 1928; Brug and de Rook, 1930). The pioneering work of Brug was acknowledged in 1958 when Buckley proposed the new genus Brugia and F. malayi was re-named as Brugia malayi [10]. In the 1960's another filarial species was discovered in Portuguese Timor and given the name Microfilaria timori [11]. Partono confirmed that the new species belonged to the genus Brugia and called it B. timori [12].

**Global Distribution of Lymphatic Filariasis**

Over 120 million people are literally affected in 72 countries, while around 20% of the world's population is living at the risk of the disease (WHO, 1997a). The nematode parasite W. bancrofti is the major cause of this disease accounting for 90% of the cases. India alone contributes about 40% of the total global burden of this disease [13] and there are approximately 21 million people with symptomatic filariasis and 27 million who have asymptomatic microfilaraemia [13,14,15]. It has also been estimated that economic loss to India is to the tune of US $ 840 million [13]. In India W. bancrofti and B. malayi are the only two filarial species causing lymphatic filariasis and the former being the major contributor and the latter is endemic to a few parts of Kerala, Tamil Nadu, Andhra Pradesh, Orissa, West
Figure 1. Distribution of Lymphatic Filariasis, Worldwide, 2012

Figure 1 Geographic map of the tropical and subtropical regions of the world endemic for lymphatic filariasis (Image available at http://www.filariasis.org)
Figure 2. Life Cycle

Figure 2. Diagram of the life cycle of Brugia malayi, shown alternating between the human host and mosquito vector. During a blood meal, an infected mosquito introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound (1) and then migrate to the lymphatics (2). Female worms produce millions of microfilariae, which circulate in the blood until being ingested by a mosquito taking a blood meal (4). After ingestion, the microfilariae develop to the L3 stage in the mosquito gut before migrating to the proboscis, primed to infect another human when the mosquito takes a blood meal. (Image available at http://www.dpd.cdc.gov)
Bengal, Assam, and Madhya Pradesh (Raina et al., 1995). In India, a total of 554.2 million people are at risk of infection in 243 districts (National Health Policy 2002).

*W. bancrofti* accounts for about 98% of the national burden and is widely distributed in 17 States and Union Territories (Figure 1). An overview of the traditional endemic foci shows that the concentration of infection happens mainly around the river basins and eastern and western coastal plains of India [15]. In addition approximately 16 million people have lymphedema or elephantiasis along with the recurrent episodes of acute adenolymphangitis (ADL). Lastly, around one million individuals have cryptic infections resulting in conditions such as tropical pulmonary eosinophilia (TPE) (WHO, 2002). According to a WHO report, India, Indonesia and Bangladesh alone contribute >70% of the infection worldwide. Apart from these countries, six other countries, such as Maldives, Myanmar, Nepal, Sri Lanka, Thailand and Timor-Leste are the other endemic countries having more number of lymphatic filarial cases.

**Life Cycle**

The three species of lymphatic dwelling filariae have a complex life cycle that alternates between the mosquito vector and the human host. *W. bancrofti* accounts for approximately 90% of all infections while *B. malayi* and *B. timori* collectively make up the other ~10% mostly in Southeast Asia and the Pacific. The parasite’s life cycle (Figure 2) consists of dioecious male and female adult worms, the microfilaria stage, and four larval stages (L1-L4). The third larval stage (L3) is the infectious stage and is transmitted to humans via a mosquito intermediate host. Upon entry into the human host, the L3 larvae migrate to the nearest afferent lymphatics, molt to the fourth larval (L4) stage and undergo a final molt into sexually mature adults. After sexual reproduction, adult females produce millions of sheathed microfilariae (mf) that migrate to and circulate in the bloodstream, usually in synchrony with
diurnal mosquito feeding patterns [16] (Scott, in *Lymphatic Filariasis*, 2000). During subsequent blood meals the mosquitoes would then pick up the mf. In the abdomen of the mosquito the mf maturing into the first larval stage (L1) and then undergo two molts becoming second larval stage (L2) and subsequently emerging as human infective L3 larvae [17] (Scott in *Lymphatic Filariasis*, 2000).

From beginning to end the duration of the *B. malayi* lifecycle is as follows: in the mosquito gut, the molt from L1 to L2 ranges from six to ten days and the molt from L2 to L3 takes one to three days. Thus, it takes approximately two weeks for the infective L3 larvae to mature in the vector. Subsequent to a bite from an infective mosquito and the entry of L3s into human skin, the L3 would transform into L4 after nine to fourteen days in the human host, while the worm is migrating through the circulatory system to the lymphatics. The final molt, from the L4 stage to the adult worm, requires a minimum of three months but may last as long as twelve months and is localized in the lumen of dilated lymphatic vessels, the final site of the adult worm in the human body [17]. *W. bancrofti* adults are typically found in the lymphatic vessels of the lower extremities in females and the lymphatic vessels of the spermatic cord in males. Although the life span of adult worms is not precisely known, it is estimated that adult females can remain reproductively active on the order of 5 years [16].

**Diagnosis and Treatment**

Diagnosis of lymphatic filariasis depends upon the detection of the parasite or parasite antigens in the blood. The classical “gold-standard” technique has been used in detecting microfilaria in a peripheral blood sample drawn during the time of peak parasitemia (usually at night); however, this technique is now considered to be quite insensitive and underestimate the true prevalence of infection in an area. Recently a highly sensitive and specific test that
detects the presence of an adult worm antigen in the blood of actively infected individuals has been developed to diagnose *W. bancrofti* infection [18,19]. This test is useful because antigen levels remain constant throughout the day and it has the added advantage of being able to detect single-sex and non-fecund infections in which microfilaria are not produced. Serologic enzyme immunoassay tests including anti-filarial IgG1 and IgG4 provide an alternative to microscopic detection of microfilariae for the diagnosis of lymphatic filariasis. Patients with active filarial infection typically have elevated levels of anti-filarial IgG4 in the blood and these can be detected using routine assays.

Filarial infection is a significant cause of disability in the tropical areas of the world and the chronic pathologies of lymphatic filariasis are physically debilitating, economically costly and socially stigmatizing [2]. Once the disease has progressed to the elephantiasis stage, it is irreversible (even if there is no active infection) but the infection burden in endemic communities can be reduced with the effective chemotherapeutic agents ivermectin, albendazole and diethylcarbamazine (DEC) [16]. Two drug combinations, the most commonly used (DEC 6mg/Kg) and albendazole (400mg) effectively kill the circulating microfilariae and greatly reduce the fecundity of adult worms [4]; this in turn reduces the uptake of microfilariae by the mosquito vector and thereby disrupts transmission in the endemic communities. Due to the development of these effective treatments within the last two decades, in addition to improved diagnostic methods for monitoring infection rates, global eradication of lymphatic filariasis has been seriously considered as a feasible public health venture by the WHO [20].
The Spectrum of Filarial Disease

Lymphatic filariasis can manifest itself in a variety of clinical and subclinical conditions. Traditionally, it has been accepted that people living in an endemic area can be classified into five groups: (1) uninfected but exposed; (2) clinically asymptomatic, infected; (3) those with acute filarial disease with or without microfilaremia; (4) those with longstanding chronic infection associated with pathological conditions; and (5) those with tropical pulmonary eosinophilia (TPE).

Endemic Normals (EN/UN)

Uninfected, but exposed individuals (asymptomatic amicrofilaremia or endemic normals)

In endemic areas a considerable proportion of the population remains uninfected despite exposure to the parasite [WHO Report 1992]. This group has been termed endemic normals. The incidence of endemic normals in a population ranges from 0 % to 90 % in different endemic areas (Wkly Epidemiol Rec 2007).

Asymptomatic Individuals (INF)

Subclinical (or asymptomatic) patent infection (with or without microfilaremia)

In areas endemic for lymphatic filariasis many individuals exhibit no symptoms of filarial infection and yet on routine blood examinations demonstrate the presence of significant number of parasites or the presence of circulating parasite antigen (a surrogate for viable adult worms). These individuals are carriers of infection (and for those that are microfilaria+ the reservoir for ongoing transmission). The burden of parasites in these individuals can reach dramatically high numbers exceeding 10,000 microfilariae in 1 ml of blood. With the availability of imaging techniques (e.g. ultrasound, lymphoscintigraphy, MRI,
CT) it has become apparent that virtually all persons with microfilaremia have some degree of subclinical disease; these include marked dilatation and tortuosity of lymph vessels with collateral channeling, increased flow, abnormal patterns of lymph flow [21,22]; scrotal lymphangiectasia [23]; and microscopic hematuria and/or proteinuria [24]. Thus, while apparently free of overt symptomatology, the subclinical patently infected individuals are clearly subject to subtle pathological changes.

**Chronic Pathology (CP)**

The chronic sequelae of lymphatic filariasis develop years after initial infection [25]. In Bancroftian filariasis the main clinical features are hydrocele, lymphedema, elephantiasis and chyluria. The manifestations are hydrocele and swelling of the testis and/or lymphedema of the entire lower limb, the scrotum, the entire arm, the vulva, and the breast [25]; on the other hand, in Brugian filariasis the leg below the knee and the arm below the elbow are commonly involved but not the genitals. Therefore, the development of pathology is thought to be dependent on the presence of the adult worm. Histologically, the worm elicits little reaction as long as it is alive; however, upon death of the adult worm, a granulomatous reaction ensues [26]. The granulomas are characterized by macrophages (which develop into giant cells), plasma cells, eosinophils, neutrophils and lymphocytes. There is endothelial and connective tissue proliferation with tortuosity of the lymphatics and damaged or incompetent lymph valves. This typically results in lymphatic dilatation and subsequently lymphatic dysfunction and compromise leading to lymphedema. Early pitting edema can give rise to subsequent brawny edema with hardening of tissues and later hyper-pigmentation and hyperkeratosis with wart-like protuberances, which, on histological examination, reveal dilated loops of lymphatic vessels within nodular lesions. Very important in the progression of these lesions is the fact that redundant skin folds, cracks and fissures in the skin provide havens for bacteria and fungi.
to thrive and intermittently penetrate the epidermis leading to either local or systemic infections. Sometimes the skin over the nodules breaks down causing the dilated lymphatic within to rupture and discharge lymph fluid directly into the environment, at the same time serving as a pathway for entry of microorganisms into the lymphatic [27].

In men scrotal hydrocele is the most common chronic clinical manifestation of bancroftian filariasis [23,24]. It is uncommon in childhood but is seen more frequently post puberty and increases in incidence with age. In some endemic communities, 40–60 % of all adult males have hydroceles. Hydroceles are due to accumulation of edematous fluid in the cavity of the tunica vaginalis testis. Though the mechanism of fluid accumulation is unknown, direct ultrasonographic evidence indicates that in bancroftian filariasis the scrotal lymphatics are the preferred site of localization of the filarial worms and their presence may stimulate not only the proliferation of lymphatic endothelium but also a transudation of hydrocele fluid whose chemical composition is not dissimilar to serum. Chronic epididymitis and funiculitis can also occur. The prevalence of chyluria (excretion of chyle) is very low.

Other manifestations

Lymphatic filariasis has been associated with a variety of renal abnormalities including hematuria, proteinuria, nephrotic syndrome and glomerulonephritis [28]. Circulating immune complexes containing filarial antigens have been implicated in the renal damage. Lymphatic filariasis may also present as a mono-arthritis of the knee or ankle joint [29].

Innate Immune Response to Filariasis

The innate immune response is able to sense invading microorganisms and react rapidly to contain infection-allowing time for the more sophisticated adaptive immune system to develop. Specialized receptors present on cells of the innate immune system interact with molecular patterns specific to microorganisms. In terms of innate immune responses, a recent
work has indicated that some helminth infections may also be associated with an altered innate immune response. A well-known family of such receptors is the toll like receptor (TLR), which initiate a cascade of signals leading to release of cytokines and chemokines that set the anti microbial events into action [28]. Recently, specific helminth derived molecules have been identified that can stimulate the innate immune system via TLR [9,29,30]. Chronic infections with schistosomes and filarial parasites have been shown to result in a lower responsiveness of monocytes or B cells to TLR ligands [31,32]. Thus, helminth infections not only act to down regulate adaptive immune responses but also seem to interfere with the innate immune system.

Innate immune responses also play a prominent role in the development of pathology as evidenced by the occurrence of lymphatic damage in animal models of filarial infection lacking an adaptive immune system [30]. Filarial pathology arises out of a complex early interplay between the parasite and the host’s innate responses and its tissue homeostasis. Common sequelae of the stimulation of the innate response include the production of vascular endothelial growth factors, e.g. by macrophages [31]. The VEGF family member that has been implicated to play a role in filarial disease is VEGF-A [35]. These VEGF factors are elevated following LF infection with different levels of expression in the different disease forms [32]. Vascular endothelial growth factor (VEGF) family members have also been implicated in lymphangiogenesis. It was recently shown that lymphatic-endothelial specific VEGF-C levels are significantly elevated in individuals with filarial disease [37]. Increased circulating levels of VEGF-C may not be confined to individuals with overt pathology since filarial-infected individuals with subclinical disease also exhibit elevated levels of this factor [38].

Innate cytokines appear to play a prominent role in the initiation of pathology in
filarial-infected animal models. The importance of pro-inflammatory cytokines, possibly of innate origin in the pathogenesis of lymphedema, has been further strengthened by a series of studies in humans in either the early or late stages of lymphedema. Pro-inflammatory cytokines of innate origin also appear to play an important role in brugian infection since infection of nude mice results in elevated levels of IL-1, IL-6, TNF-α and GM-CSF in lymph fluid [32]. In human studies they have shown that pro-inflammatory cytokines such as TNF-α, IL-6 and soluble TNF receptor plays the role in chronic pathology [33]. Studies have shown that individuals with chronic lymphatic pathology have 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 the peripheral circulation. Similarly, while patients with both acute and chronic manifestations of LF have elevated circulating levels of IL-6 and IL-8, only those with chronic disease manifestations have elevated levels of sTNF receptors [40].

Another important mechanism of immune activation in chronic infections is the occurrence 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. Apart from this systemic immune activation, progressive fibrosis and extracellular matrix remodeling is another salient 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) are produced by a variety of cell types including macrophages, granulocytes, epidermal cells, and fibroblasts. The dysregulation of MMPs and TIMPs is
Figure 3. T Cell Differentiation

Figure 3. T helper cell differentiation is classically regarded as a dichotomy between two main cell types, termed Th1 and Th2. Th1 cells produce IFN-γ as their signature cytokine and are predominantly involved in cell-mediated immunity against intracellular pathogens, through activation of macrophages. In contrast, Th2 cells do not produce IFN-γ and their signature cytokines are IL-4, IL-5 and IL-13. Th2 cells are the most effective activators of B cell proliferation and antibody production, thereby mediating humoral immunity essential for the eradication of extracellular pathogens. More recently, Th17 cells have been described as a distinct T helper subset characterized by the production of IL-17A, IL-17F and IL-22, contributing to host defense against extracellular pathogens particularly at mucosal surfaces.

(Image available at http://www.erasmusmc.nl/content/3025942/3030149/thelper17subsetinpulmdiseases?lang=en)
known to underlie the development of pathology in several infections including viral, bacterial, spirochetal, protozoan, fungal and parasitic infections. Another study has also examined the alterations in pro-fibrotic factors in filarial pathology and revealed that increased levels of basic fibroblast growth factor (bFGF) and placental growth factor (PIGF) can also occur in filarial lymphedema patients [38]. Studies have shown that individuals with chronic lymphatic pathology have elevated levels of C-reactive protein (an acute phase protein, indicating an acute inflammatory response) [34]. Inflammatory adverse reactions also occur in people treated with anti-filarial drugs and can be severe in those with large numbers of parasites [42]. Systemic inflammation is associated with increase in IL-6, IL-10, lipopolysaccharide-binding protein (LBP) and soluble TNF α receptors with IL-6 and LBP particularly associated with severe reactions.

**Adaptive Immune Response in Lymphatic Filariasis**

**T cell Differentiation**

Adaptive immune responses are in general required for protection against many if not most pathogens. CD4+ T cells are the key component of adaptive responses to both intracellular and extracellular pathogens. The major function of CD4+ (helper) T cells is to provide help to other lymphocytes mounting an efficient immune response. By secreting appropriate cytokines and expressing a variety of co-stimulatory molecules, CD4+ T cells are required for the generation of high affinity antibody responses to pathogens and for the formation of long-lived plasma cells and memory B cells [35].

Naïve CD4+ T cells differentiate (Figure.3) into various subsets upon interaction with an antigen presented by the professional antigen-presenting cells (APCs) such as dendritic cells (DC). CD4+ T cells require 3 signals for their lineage commitment (Kenneth et al.,
The first signal is generated following the interaction between T-cell receptor (TCR) and the peptide presented in the context of major histocompatibility complex (MHC) Class II on an APC [36]. The second signal is generated following the interaction between the CD28 co-receptor on the T cell and B7 family of co-stimulatory molecules such as CD80 or CD86 on the APC. The third signal is generated by inflammatory cytokines produced by the APC or other cells at the site of T cell activation. These cytokines direct differentiation of naïve CD4+ T cells into a particular effector subset. Effector CD4+ T cells can be categorized into three major subsets based on the type of cytokine they produce and the major transcription factor (TF) they express. Two other subsets of CD4+ T cells have also been identified; Tregs express TF FoxP3; these cells secrete anti-inflammatory cytokines like TGF-β and IL-10. Tregs maintain immune homeostasis by limiting the magnitude of immune response against pathogens and control inflammatory reactions [37]. T follicular helper cells (Tfh) express a TF Bcl-6 and these cells are essential for the production of high affinity IgG antibodies [35]. Existence of Th9 and Th22 subsets has also been recently suggested [46,47].

**Th2 Cytokines**

Th2 cell differentiation requires the cytokine IL-4 and is controlled by master transcription factors GATA3 and STAT6. Th2 immune responses are characterized by the production of IL-4, which can serve as an autocrine factor for Th2 differentiation and can stimulate activated B cells and promote differentiation of B cells into plasma cells. Th2 cells also produce IL-5, a key mediator of eosinophilopoiesis and eosinophil activation. IL-13, a product of Th2 cells, has some overlapping functions with IL-4. Th2 cells fail to produce IFN-γ and produce the signature cytokines IL-4, IL-5 and IL-13.

A variety of different cell types can make IL-4 (T cells, eosinophils, basophils, mast
cells, natural killer (NK) cells and some antigen-presenting cells (APCs)). IL-4 plays a central role in the differentiation of antigen-stimulated naive T cells into Th2 cells [38]. The presence of IL-4 during in vitro priming determines the cytokine-producing potential of CD4+ T cells from T cell receptor transgenic mice [39]. IL-4 has a central role in the regulation of allergic conditions. It is the major stimulus for Th2 development and suppresses Th1 development. Furthermore, IL-4 is important in the control of immunoglobulin class switching. It determines that human B cells switch to IgE and IgG4 and mouse B cells to IgE and IgG1. IL-4 plays a major role in the development of protective immune responses to helminths and other extracellular parasites. [40].

IL-5 secretion is mainly controlled via the transcription factor GATA-3 that also acts as a transcription factor for IL-4 and IL-13. IL-3, GM-CSF and IL-5 synergize for differentiation and function of myeloid cells. In general, IL-5 leads to growth, activation, mobilization, differentiation and survival of eosinophils [51,52,53]. Moreover, IL-5 displays eosinophil chemotactic activity, increases eosinophil adhesion to endothelial cells, and thereby enhances effector functions of eosinophils.

T cells, mast cells, basophils, eosinophils, and NKT cells all of them produce IL-13. Major target cells are B cells, mast cells, epithelial cells, eosinophils, smooth muscle cells; and macrophages IL-13 is antagonized via the Th1-type cytokines IFN-γ, IL-12, IL-18, and TNF-α and the regulatory cytokine IL-10 [41]. IL-13 can induce class-switching to IgG4 and IgE in combination with CD40 stimulation. [42].

Th2 responses are considered to be the hallmark of helminth infection and are indeed required for host resistance to a variety of helminths in animals [43]. The cytokines IL-4, IL-5, and IL-13, secreted by Th2 cells, provide protective immunity in the context of parasite
infection [57,58], but also initiate, amplify, and prolong allergic responses by enhancing production of IgE and are also responsible for recruitment, expansion, and differentiation of eosinophils and mast cells [59,60,61,62]. The canonical host immune response to filarial parasites is of the Th2 type and involves the production of cytokines—IL-4, IL-5, IL-9, IL-10, and IL-13; the antibody isotypes—IgG1, IgG4 and IgE expanded populations of eosinophils, basophils, mast cells and alternatively activated macrophages. Th2 responses induced by filarial parasites is a stereotypical response of the host; its initiation requires interaction with many different cell types most notably: (1) stromal cells; (2) dendritic cells and macrophages; (3) eosinophils; (4) mast cells; (5) basophils and (6) epithelial and innate helper cells [43].

Human filarial infection is known to be associated with augmented Th2 responses (King C.L.1991). Thus, in human lymphatic filariasis (LF) patent filarial infection is associated with an antigen – specific expansion of Th2 cells (mostly defined by IL-4 expression) and enhanced production of IL-4 and IL-13 [44]. Th2-type immune responses are composed of three major features [43]: immunosuppression, immunological tolerance and modified Th2 response. Th2 responses are effective in helping B cells to produce antibodies and are important in combating extracellular bacteria or parasites in the induction of humoral immunity [33, 64, 65, 66, 67].

Th9 Cytokines

Th9 cells are one of the more recently described subsets of effector T cells. They develop from naive T cells in the presence of transforming growth factor β (TGF-β) and interleukin-4 (IL-4). As the sobriquet would suggest, cells cultured under these conditions are primed for the production of IL-9 and require transcription factors that include STAT6 (signal transducer and activator of transcription 6), PU.1, IRF4 (interferon response factor 4) and
GATA3. In addition to IL-9, Th9 cells have been shown to produce IL-10 and IL-21 although these are not regulated coordinately with IL-9 and their role in Th9 cell function is unclear [68]. The transcription factor PU.1 is required for the development of IL-9-producing T cells and allergic inflammation. [46,69]. IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-β, generates IL-9+ IL-10+ Foxp3(-) effector T cells [45,46,47,48].

Th9 lymphocytes are pro-inflammatory cells that work in a broad spectrum of autoimmune diseases and in allergic inflammation. In mouse, Th9 cells induce inflammation in T cell transfer colitis and in EAE model [69]. IL-4 inhibits TGF-beta-induced Foxp3 T cells and, together with TGF-β, generates IL-9+ IL-10+ Foxp3 (+) effector T cells [72]. Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes, and also contribute to allergic diseases [49]. IL-9 is highly expressed in the lungs of asthmatic patients [74] and was significantly higher in T cells from atopic infants in comparison with nonatopic group [50]. Th9 cells are a recently discovered subset of CD4+ T cells, characterized by their unique ability to produce both IL-9 and IL-10 but not IL-4 [48,49]. Traditionally associated with the Th2 response, IL-9 is a member of the common g-chain cytokine family and exerts broad effects on many cell types, including mast cells, eosinophils, T cells, and epithelial cells [76,77]. However, it has become apparent from studies in mice that many different CD4+ T cell subsets have the capacity to secrete IL-9. A subset of IL-9– producing CD4+ T cells (Th9 cells) distinct from Th1, Th2, and Th17 cells has been identified. These Th9 cells are characterized by the coincident production of IL-9 and IL-10 and develop from naive CD4+ T cells under the combined influence of IL-4 and TGF-β [46,69]. It has also been shown that IL-9 secretion of murine Th2 cells is also dependent on TGF-β and that TGF-β can redirect committed Th2 cells toward the Th9
phenotype [46]. IL-1 family members can also contribute to IL-9 production [46]. Moreover, regulatory T cells expressing IL-9 have been described as having a role in the induction of peripheral tolerance [79]. Th9 cells in humans were initially described as IL-9 cells co-expressing IL-17 [80]; however, IL-9–producing CD4+ T cells distinct from Th1, Th2, and Th17 cells have also been described recently [81,82]. In humans, Th9 cells are thought to play an important role in allergy [51], atopy [50], asthma [51], autoimmunity [52], and antitumor immunity.

**Th1 Cytokines**

Th1 cells are characterized by the production of their cytokines, notably IFN-γ, TNF-α, IL-2, lymphotoxin and granulocyte-macrophage colony-stimulating factor (GM-CSF), which prompts stimulation of Th1 cells, CTL, and maturation and activation of macrophages as well as granulocytes. The differentiation of Th1 cells requires the cytokine IL-12, the master transcription factor T-bet [85] and the signaling transducer and activator of transcription STAT4.

Th1 cells play a central role in the clearance of intracellular pathogens [53]. However, they are also responsible for mediating immune pathology and autoimmune disease in a number of settings. Human filarial infection is known to be associated with down regulation of parasite-specific Th1 responses and T cell proliferation and but with augmented Th2 responses [44]. Infection with infective larvae results into pre-patent immunological signs such as enhanced filaria specific T-cell proliferation, formation of lymphatic granuloma; increased production of Th1 cytokines such as IL-2 and IFN-γ by T cells and presence of antibodies to microfilarial sheath [54]. When the maturation and development of larvae results into the establishment of adult worms, the above immunological features are rapidly down
regulated. The antigen-presenting cells and IL-4 and IL-10 activity suppress the induction of Th1 cytokines and filarial specific T-cell proliferation leading to rapid induction of anti-inflammatory cytokines such as IL-4. Recently it has been demonstrated that following the immediate entry of L3, there is a dominance of pro-inflammatory cytokines and up-regulation of activated T cells surface markers CD69 and CD71, with significant increase in the frequency of T cells expressing pro-inflammatory Th1 cytokines like IFN-γ, TNF-α, GM-CSF, and IL-8 [88]. The individuals with asymptomatic microfilaraemia have a lower secretion of IFN-γ-secreting cells than the individuals with chronic pathology [55]. Studies in filaria-infected humans have shown that peripheral blood mononuclear cells (PBMC) stimulation with filarial antigens or polyclonal activators results into IL-4, IL-5 and IL-10 production [90, 91] with concomitant inhibition/suppression of IFN-γ responses [56].

**Th17 Cytokines**

Th17 CD4 T cells have been characterized to produce the cytokines IL-17A (IL-17) and IL-17F, as well as IL-21 and IL-22. The differentiation of Th1 or Th17 cells occurs following exposure to APC-derived polarizing cytokines such as IL-12 [57]; generation of human Th17 cells is dependent on IL-23, IL-1β, [58, 59] TGF-β [59] and IL-6 [58]. These polarizing cytokines further induce the expression of the transcription factors T-bet or RORγt and RORα for Th1 and Th17 differentiation respectively [60, 61].

During Th17 differentiation, human naive T cells must be exposed to IL-1β, IL-6, IL-23, and TGF-β before they express maximum levels of IL-17 [95]. Th17 lymphocytes, beyond their protective role in the clearance of extracellular pathogens, also play a role in the pathogenesis of several autoimmune and inflammatory [62]. Th1 and Th17 cells can be involved in the pathogenesis of human autoimmune and inflammatory disorders and that these
two cell subsets can develop from the same precursors and coexist in the same microenvironment [96,97]. Th17 has shown to be associated with the development of pathology in schistosomiasis [98,99], leishmaniasis [100,101] and toxoplasmosis. IL-17 also plays an important role in regulation of inflammation and fibrosis. [102]. Th22 cells that express IL-22 are thought to play an important role in protection against intestinal pathogens [103] and an anti-inflammatory role in SLE and RA [52,63].

**IL-10 family of Cytokines**

The six members of the IL-10 family can be divided into two groups with respect to their cellular sources. IL-10, IL-22, and IL-26 form the first group and are preferentially produced by immune cells. In contrast, IL-19, IL-20, and IL-24 can be secreted both by tissue cells and immune cells [64]. Interleukin (IL)-10 is a pleiotropic, immunoregulatory cytokine that is important in protecting the host from infection-associated immunopathology, autoimmunity, and allergy. IL-10 was initially characterized as a Th2 specific cytokine [65] however; further investigations revealed that IL-10 production was also associated with T regulatory (Treg) cell responses [66,67,68,69]. It is now known that almost all cells of both the innate and adaptive arms of the immune system can express IL-10, including dendritic cells (DC), macrophages, mast cells, natural killer cells (NK), eosinophils, neutrophils, B cells, CD8+ T cells, and Th1, Th2, and Th17 CD4+ T cells [66,68,69,70,71,72,73,74,75]. Several studies of human autoimmune disease have revealed that the level of IL-10 detected in patient samples correlates inversely with disease severity [115,116,117,118,119].

IL-10 is a powerful immune-regulatory cytokine known to be induced in a variety of helminth infections [76]. The IL-10 dominated regulatory environment induced in chronic helminth infections is known to modulate the entire repertoire of CD4+ and CD8+ T cell effector functions [76]. Increased levels of spontaneous as well as parasite – specific IL-10 are
associated with filarial infections [121] and thought to play a crucial role in down-regulation of T cell-mediated immune responses [76]. Therefore, this IL-10 dominated response has the potential to regulate not only the balance of T cell subsets, but also to modulate the response to both bystander antigens and allergens as well [122,123,124]. IL-10 family cytokines elicit diverse host defense responses during infections and are known to facilitate the tissue-healing process in infection and inflammation [77]. IL-19 has been shown to be increased in both Th1- and Th2-dominant diseases such as psoriasis and asthma, respectively [126,127], data from IL-19 deficient mice and human data autoimmune diseases and other inflammatory conditions suggest that IL-19 has a potent anti-inflammatory activity [128,129]. IL-24, alternatively, has been predominantly studied in tumor immunology and has exhibited great promise as an anti-tumor therapeutic cytokine [130]. S. aureus, clearly reveals an important immuno-modulatory role for IL-19 and IL-24 in suppressing IL-1β and IL-17 dependent effector pathways and promoting susceptibility to infection [131].