CHAPTER-2

1 REVIEW OF LITERATURE

Lymphatic filariasis is vector–borne parasitic disease caused by three lymphatic dwelling nematode worms living in the lymphatic system, namely *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* belong to Class: Chromadorea, Order: Spirurida, Super Family: Filarioidea and Family: Onchocercidae.

1.1 History of Filariasis

Lymphatic filariasis has been known to occur in the Nile region of Africa. Ancient monuments suggest that the disease may have been present as early as 2000BC. The proof from Indian origin about the disease was the description of signs and symptoms of filariasis in “Madhava Nidhan” in 7th Century A.D. by Madhava Karan. Artifacts from the Nok civilization in West Africa may show scrotal swelling, another characteristic of elephantiasis. The first written account of lymphatic filariasis comes from the ancient Greek and Roman civilizations. In these civilizations, writers were even able to differentiate between the similar symptoms of leprosy and lymphatic filariasis, describing leprosy as "elephantiasis graecorum" and lymphatic filariasis as "elephantiasis arabum."

1.1.1 Discovery of Symptoms (1588-1592)

During an exploration of Goa between 1588 and 1592, Jan Huygen Linschoten wrote that inhabitants were "all born with one of their legs and one foot from the knee downwards as thick as an elephant’s leg" (Chandy *et al.*, 2011). In 1849, William Prout became the first to document a condition common to lymphatic filariasis called chyluria (passage of lymph in the urine so it appears milky) a book entitled ‘On the Nature and Treatment of Stomach and Renal Diseases’ (Chandy *et al.*, 2011).

1.1.2 Discovery of Microfilariae (1863 and 1866)

In 1863, French surgeon Jean-Nicolas Demarquay became the first to record the observation of mf in fluid extracted from a hydrocoele (another common symptom of lymphatic filariasis) (Chandy *et al.*, 2011). Three years later, Otto Henry Wucherer discovered mf in urine in Brazil. However, the connection between these two discoveries was not made
until Timothy Lewis noted the occurrence of mf in both blood and urine. Lewis was also the first to make the association between these mf and elephantiasis (Lewis, 1972).

### 1.1.3 Discovery of Adult Worm (1876)

Soon after the discovery of mf, the adult worm was documented by Joseph Bancroft. The observed species was later named after Bancroft, and we now recognize it as *W. bancrofti* (Cobbold, 1877).

### 1.1.4 Discovery of Filarial Life Cycle (1877)

Patrick Manson was the first to look for an intermediate host for lymphatic filariasis mf. In 1877, he was finally able to pinpoint the mf in mosquitoes. This discovery was later applied to other tropical diseases such as malaria, and was the first discovery of an arthropod as a vector. However, Manson incorrectly hypothesized that the transmission occurred when the mosquito deposited the filaria in water that then infected humans through ingestion of contaminated water or direct skin penetration (Cobbold, 1877).

### 1.1.5 Discovery of Transmission (1900)

In 1900, George Carmichael Low discovered mf in the proboscis of mosquitoes, and finally pinpointed that transmission is due to an infective bite from a mosquito vector (Chandy et al., 2011).

### I. Filariasis - One of the major public health problems in tropical regions

Family of filarial parasite filarioidea, includes approximately 500 species. Except fishes almost all the species of animal kingdom are affected with filarial infections. They are parasitic in nature and reside in lymphatics, connective tissues, body cavity etc. of vertebrate host. The infection is transmitted by blood sucking arthropod host. Filariasis has afflicted people in the tropical areas of the world for thousands of years but even up to comparatively recent times it has been poorly understood and its importance under recognized. In the last 2 decades or so there has been a flurry of activity in filariasis research, which has provided new insights into the global problem of filariasis, the pathogenesis of filarial disease, diagnosis and control.

LF is synonymous to elephantiasis, is prevalent in many parts of the tropics and subtropics of the world. Globally, about 1100 million people live in areas endemic for lymphatic
filariasis. An estimated 120 million people in 73 countries are currently infected, and an estimated 1.393 billion live in areas where filariasis is endemic and mass drug administration (MDA) is required. LF is the second leading cause of chronic disability worldwide due to its stigmatizing and disabling clinical manifestations, including 15 million people with lymphoedema (elephantiasis) and 25 million men with urogenital swelling, principally scrotal hydrocele (WHO, 2012), http://www.who.int/wer/. In India, more than 500 million people are exposed to infection with 45.5 million people showing circulating mf and another 22.5 million people suffering from chronic manifestations of the disease. Around 50% of the infected persons suffer from acute episodic attacks of adenolymphangitis and fever (Pani et al., 2002). Tolerability and efficacy of single dose albendazole, diethylcarbamazine citrate (DEC) or co-administration of albendazole with DEC in the clearance of W. bancrofti in asymptomatic microfilaraemic volunteers in Pondicherry, South India: a hospital-based study.

**Table 2.1:** Common human filarial parasites: Geographical distribution and clinical syndrome

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Geographical distribution</th>
<th>Vector</th>
<th>Seat of predilection</th>
<th>Mf nature</th>
<th>Common symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Wuchereria bancrofti</em></td>
<td>West Indies, North America</td>
<td><em>Culex quinquefasciatus</em></td>
<td>Lymphatics</td>
<td>Blood and sheathed (nocturnal periodic)</td>
<td>Elephantiasis</td>
</tr>
<tr>
<td><em>Brugia malayi</em></td>
<td>Malaysia, India</td>
<td><em>Mansonia sp</em></td>
<td>Lymphatics</td>
<td>Blood and sheathed (periodic and sub-periodic)</td>
<td>Elephantiasis</td>
</tr>
<tr>
<td><em>Brugia timori</em></td>
<td>Indonesia</td>
<td><em>Anopheles barbirostris</em></td>
<td>Lymphatics</td>
<td>Blood and sheathed (nocturnal periodic)</td>
<td>Adenolymphangitis</td>
</tr>
<tr>
<td><em>Onchocerca volvulus</em></td>
<td>Africa, Central America</td>
<td><em>Simulium sp.</em></td>
<td>Subcutaneous nodules</td>
<td>Skin and unsheathed and non-periodic</td>
<td>Dermatitis, River blindness, Skin nodules</td>
</tr>
<tr>
<td><em>Loa loa</em></td>
<td>West &amp; Central Africa</td>
<td><em>Tanabid</em></td>
<td>Migrating cutaneous &amp; subcutaneous tissues</td>
<td>Blood sheathed and diurnal periodic</td>
<td>Calabar swelling</td>
</tr>
<tr>
<td><em>Acanthocheilonema perstans</em></td>
<td>Africa, South America</td>
<td><em>Culicoid sp.</em></td>
<td>Body cavity</td>
<td>Blood unsheathed and non-periodic</td>
<td>Usually benign</td>
</tr>
<tr>
<td><em>Acanthocheilonema streptocercum</em></td>
<td>Africa</td>
<td><em>Culicoid sp.</em></td>
<td>Skin and Subcutaneous</td>
<td>Skin, non-periodic and unsheathed</td>
<td>Benign</td>
</tr>
<tr>
<td><em>Mansonella ozzardi</em></td>
<td>South America</td>
<td><em>Culicoid sp.</em></td>
<td>Body cavity</td>
<td>Blood unsheathed and non periodic</td>
<td>Usually benign</td>
</tr>
</tbody>
</table>
1.2 Causative agent

I. *Wuchereria bancrofti*

*W. bancrofti* is the most well-documented and widespread cause of lymphatic filariasis. It is more common to find elephantiasis in patients affected with *W. bancrofti* than those affected with the Brugian filariasis, although it can occur. Bancroftian filariasis characteristically includes symptoms associated with the genitalia or chyluria in heavily infected patients (John *et al.*, 2006). *W. bancrofti* is transmitted mainly by *Anopheles* and hence microfilaria of *W. bancrofti* is found at high levels at night. Additionally, *W. bancrofti* has no known animal reservoir except humans.

The mf, or larval stage of *W. bancrofti*, are sheathed, and range from approximately 245 to 300 µm. Nuclei do not appear at the end of the tail, which is a major difference from other mf. It can take several months for the mf to sexually mature, and in the adult stage they can live for several years. As adults, the males range from 2.5 to 4 cm, and the females range from 5 to 10 cm. One end of the round body is blunt, while the other is pointed. Both Bancroftian and Brugian filariae lack a digestive system, instead absorbing nutrients from their hosts (John *et al.*, 2006).

II. *Brugia malayi*

The distribution of *B. malayi* is very similar to that of *W. bancrofti*. However, cases are concentrated in Asia, including South China, India, Indonesia, Thailand, Vietnam, Malaysia, the Philippines, and South Korea. Brugian filariasis does not include symptoms associated with the genitalia or chyluria. *B. malayi* is transmitted by *Mansonia* mosquitoes. Since these mosquitoes feed primarily during the day, *B. malayi* mf can be found in the blood during the day. *B. malayi* has been found in Macaques, leaf monkeys, cats and civet cats.

The morphology is like to *W. bancrofti*. Generally, mf range from 200 to 275 µm and adult-female worms average about 3.5 to 6 cm long while male are around 1.5 to 2.1 cm. Mf of *B. malayi* are sheathed like *W. bancrofti*, and have a very similar shape. However, the nuclei extend nearly to the tip of the tail, a characteristic not shared with *W. bancrofti* (John *et al.*, 2006).
III.  **B. timori**

*B. timori* is the least common and therefore least studied species of filaria known to cause lymphatic filariasis. This species was reported on the island of Timor in 1964, and has since been found in other islands in Indonesia. In regards to vectors, periodicity and reservoirs, *B. timori* is more similar to *W. bancrofti* than to *B. malayi*. Transmission of *B. timori* is by the *Anopheles barbirostris*, a vector that feeds at night. As a result, high levels of *B. timori* mf are found in the blood at night. *B. timori* also has no known animal reservoir.

In regards to symptoms and morphology, *B. timori* resembles *B. malayi* more than *W. bancrofti*. Like *B. malayi*, symptoms associated with *B. timori* are similar to *W. bancrofti*, with elephantiasis only expressed in the lower part of the limbs. Mf of both *B. timori* and *B. malayi* has nuclei that extend to the tip of the tail. However, at approximately 310 µm, *B. timori* microfilaria is slightly larger than that of *B. malayi* (John et al., 2006).

**Table 2.2**: Difference between causative agents of lymphatic filaria in different categories

<table>
<thead>
<tr>
<th>Categories</th>
<th><em>W. bancrofti</em></th>
<th><em>B. malayi</em></th>
<th><em>B. timori</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>Only human</td>
<td>Human &amp; animals</td>
<td>Human &amp; animal</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Genitalia &amp; chyluria</td>
<td>Uncerated nodules</td>
<td>Genitalia &amp; chyluria</td>
</tr>
<tr>
<td>Transmission</td>
<td>Anopheles/Culex</td>
<td><em>Mansonia mosquito</em></td>
<td><em>Anopheles barbirostris</em></td>
</tr>
<tr>
<td>High mf count</td>
<td>In night</td>
<td>In day</td>
<td>In night</td>
</tr>
<tr>
<td>Mf size</td>
<td>245-300  m</td>
<td>200-275  m</td>
<td>300-310  m</td>
</tr>
<tr>
<td>Male size</td>
<td>2.5-4.0 cm</td>
<td>1.5-2.0 cm</td>
<td>1.5-2.0 cm</td>
</tr>
<tr>
<td>Female size</td>
<td>5.0-10.0 cm</td>
<td>3.5-6.0 cm</td>
<td>3.5-6.0 cm</td>
</tr>
</tbody>
</table>
1.3  Life cycle of *B. malayi*

Like other filarial nematodes, *B. malayi* develops through four larval stages into an adult male or female (Fig. 2.1), entirely within two host—a mosquito vector (*Culex, Aedes*, and *Anopheles*) and humans (or rodent in case of experimental filariasis). Mosquitoes are intermediate host, they provide environment for development of mf into L₃ and man is a definitive host because it facilitates reproductive phase of the parasite.

1.3.1 Development in intermediate host (mosquito)

Mosquitoes suck blood along with mf for there meal in night. These mf (L₁) shed their sheath and penetrates the stomach wall to migrate to thoracic muscles where they settle down and take two moult to L₃.

The slender active mf transforms into the short thick inactive sausage stage larvae, which has a slender tail (characteristic feature). After first moult the L₁ become short tailed L₂ larva with 1 or 2 papillae at the caudal end. Followed by second moult the L₂ grows to infective (L₃) stage larva which measures between 1500 – 2000 μm in length and 23 – 28 μm in breadth with characteristic 3 ill defined caudal papillae. Infective larvae migrate to the abdomen and then to head and proboscis. At this stage L₃ can escape to infect fresh host while mosquitoes take blood meal (Life cycle of *Brugia malayi*. The Centers for Disease control. http://www.dpd.cdc.gov/dpdx/HTML/Frames/A/Filariasis/body_Filariasis_bmalayi.htm#Geographic%20D).

1.3.2 Development in definitive host (humans or rodents)

L₃ are released in to host during blood feeding. These active larvae quickly migrate and reach to nearest lymphatics and settle down. They moult twice and develop into L₄ and L₅ stages followed by young adult stage and finally mature into male and female worms. After fertilization the female worms (which are ovoviviparous) give out young ones called mf, which pass through lymphatic duct to various systems, pulmonary capillaries and to the peripheral circulation. The mean pre-patent period i.e. from entry of L₃ to first appearance of detectable level mf in the peripheral blood is estimated to be about 9 - 12 months in case of *W. bancrofti* and 3 - 4 months in case of *B. malayi* (Life cycle of *B. malayi*: The Centers for Disease
1.3.3 Periodicity

*Brugia malayi* mf show nocturnal and diurnal periodicity during 24 hr cycles. They reside in pulmonary capillaries and a proportion of population escape from there to peripheral blood during night or day depending upon the species. The nocturnally periodic type shows a peak of mf density in the peripheral blood between 12 night and 2:00 am. Nocturnally sub-periodic and diurnally sub-periodic strains of mf circulate throughout the 24 hr with low density but their density increases during night or day, respectively (Life cycle of *Brugia malayi*: The Centers for Disease control. http://www.dpd.cdc.gov/dpdx/HTML/Frames/A/Filariasis/body Filariasis_b_malayi.htm#Geographic%20D).

![Life cycle of Brugia malayi](http://www.dpd.cdc.gov/dpdx/HTML/Frames/A/Filariasis/body Filariasis_b_malayi.htm#Geographic%20D).

**Fig. 2.1:** The life cycle of *Brugia malayi*. Vertices represent molts and edges represent lifecycle stages (Ghedin *et al.*, 2007) Science.
1.4 Epidemiology

1.4.1 Global Distribution

Lymphatic filariasis is endemic in 73 countries (WHO, 2012), mainly in the tropics; both north and south (Fig. 2.2). India, Indonesia, Nigeria and Bangladesh account for nearly 70% of lymphatic filariasis cases. Other regions include Central Africa, the Nile delta, Pakistan, Sri Lanka, Burma, Thailand, Malaysia, Southern China, the Pacific Islands, Haiti, the Dominican Republic, Guyana, Surinam, French Guiana, and Brazil (John et al., 2006).

The ‘at-risk’ population for contraction of lymphatic filariasis includes 1.2 billion people. Currently, more than 120 million people in 73 countries are affected by lymphatic filariasis, including 25 million men who suffer from the genital swellings associated with the disease and 15 million people who suffer from severe lymphoedema or elephantiasis of the leg (WHO, 2012).
1.4.2 Current situation in India

A district-level endemicity map created for India in 2000 shows that of the 289 districts surveyed up to 1995 (62% of all districts), as many as 257 were found to be endemic (Sabesan et al., 2000). Seventeen states and six Union Territories were identified to be endemic with about 553 million people exposed to the risk of infection; and of them, about 146 million live in urban and the remaining in rural areas. About 31 million people are estimated to be the carriers of mf and over 23 million suffer from filarial disease manifestations in India (WHO, 2005). The state of Bihar has highest endemicity (over 17%) followed by Kerala (15.7%) and Uttar Pradesh (14.6%). Andhra Pradesh and Tamil Nadu have about 10% endemicity. Goa showed the lowest endemicity (less than 1%) followed by Lakshadweep (1.8%), Madhya Pradesh (above 3%) and Assam (about 5%). B. malayi is prevalent in the states of Kerala, Tamil Nadu, Andhra Pradesh, Orissa, Madhya Pradesh, Assam and West Bengal. National Vector Borne Disease Control Programme, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India. http://nvbdcp.gov.in/filariasis-new.html. [Cited on 2010 Jan 13].

LF mapping by mf prevalence has been generated to depict the present scenario of human infection prevalence in India. The surveys were carried out in 443 districts out of a total 593 districts in India at different time points up to 2006 (Sabesan et al., 2000). Accordingly, the mf prevalence map at the district level is made up to the year 2006 and is shown in Fig. 2.3. Among the surveyed districts, 172 were found to be with over 1% mf prevalence. Many of these districts (58%) were detected of this status after 1990. Maximum mf prevalence (12%) was recorded from Nicobar Islands during 1996. The National level average mf rate showed a declining trend from 1.24% in 2004 to 0.63% in 2008. National Vector Borne Disease Control Programme, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India. Website: Available from: http://nvbdcp.gov.in/filariasis-new.html. [Cited on 2010 Jan 13].
1.4.3 Economic burden of disease

Estimation of socio-economic burden of the disease is important not only to understand the burden on individual patients and their families, but also for providing information crucial for developing advocacy material for mobilization of resources and commitment for its control. The economic burden of lymphatic filariasis is tremendous. Patients who are heavily infected with lymphatic filariasis have a high risk of developing chronic symptoms, including lymphodema and elephantiasis. Such symptoms can result in a decrease in productivity, as they can lead to life-threatening infections if not properly cared for, as well as mobility and functionality problems. It is estimated that India alone loses an average of $1 billion per year to lymphatic filariasis because of treatment costs and lost productivity. In endemic populations this amounts to $2 lost per patient per year, while a single dose of treatment per year costs $0.03 per person (Norris et al., 2012).

The perception upon disease by various agencies is usually based on the market value of any investment for the diseases and possible returns. Some regions of the world that can afford medicine mainly consisting of developed nations such as the U.S. and Europe generally represent the market of individuals who can pay for therapies. In contrast there are so many
more types of diseases prevailing in the developing countries and causing suffering to the humanity to a much higher extent.

1.4.4 Disability adjusted Life years (DALY)

The number of deaths is not a very informative indicator of ill health. Better is the loss of healthy life entailed by injury, disease, and premature death. The DALY or Burden of Disease statistic enables estimates to be made of the proportion of morbidity and premature mortality that can be attributed to specific risk factors. From the global burden of disease (GBD), DALY is one such measure becoming common in international comparisons (Murray & Lopez, 1996). These finding will be useful in setting up priority for filariaisis against other diseases, preparation of advocacy materials for resource mobilization, generation of political will, sensitization of policy makers, planners, programme managers, media and the community (consumers) to accept the programme and create a felt need for compliance. The DALY basically indicates the amount of healthy life expectancy lost because of a disease or risk factor, including both mortality and morbidity (Table 2.3).

Table 2.3: Contribution of Lymphatic filariasis (grouped under Tropical cluster diseases) in Global burden of diseases in terms of DALY. Source: WHO estimates for 2004 (all figures in thousands)

<table>
<thead>
<tr>
<th>Tropical Diseases</th>
<th>Cluster</th>
<th>DALY (World)</th>
<th>DALY (India)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Trypanosomiasis</td>
<td></td>
<td>1,041</td>
<td>631</td>
</tr>
<tr>
<td>Chagas disease</td>
<td></td>
<td>231</td>
<td>199</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td></td>
<td>1,021</td>
<td>686</td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td></td>
<td>1,227</td>
<td>748</td>
</tr>
<tr>
<td>Lymphatic Filariasis</td>
<td></td>
<td>4,521</td>
<td>1,420</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td></td>
<td>223</td>
<td>166</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>8,264</td>
<td>3,850</td>
</tr>
</tbody>
</table>
1.4.5 Hold in endemic countries

Nations found to be endemic tend to be tropical or subtropical due to the optimal habitat for the vectors of lymphatic filariasis. Ambient humidity is also necessary for the survival of the infective larva stage of the mf. Populations at high risk for contracting or developing a lymphatic filariasis infection are primarily poor, and a majority of the cases are concentrated in rural areas. Lymphatic filariasis is often associated with areas that have poor sanitation and housing quality (GAELF, 2006), http://www.filariasis.org/index.pl?iid=3149. Poorer, rural communities are also typically built around optimal environments for vectors, including marshes or rivers, and tend to lack the resources or capabilities to control for vectors, and transmission is high as a result.

Although there is an established high prevalence of transmission in rural areas of endemic areas, approximately 6% of urban cases of lymphatic filariasis are results of urban transmission. This percentage is high enough to confirm transmission of lymphatic filariasis in urban areas (Terranella et al., 2006).

1.5 Symptoms and pathology

Although most of the symptoms of Brugian filariasis are identical to Bancroftian filariasis, there are some differences in clinical presentation. First, Brugian filariasis tends to have a higher occurrence of ulcerated nodules, and rarely involve genital swelling or chyluria. In addition, elephantiasis is experienced almost explicitly in the lower part of the limbs, below the knee or elbow (John et al., 2006).

The nematode parasite alone is not responsible for all the symptoms; rather the host immune response against the parasite is the major one. The most severe one is the damage to the lymphatic vessels which is mediated by the immune responses to the adult worms living in them. The characteristics of these responses are inflammation (lymphangitis) of the affected area, generalized malaise and fever. Repeated episodes of lymphangitis or acute manifestations lead to the formation of fibrous and calcified tissues in and around the lymphatic vessels. These results in chronic manifestations characterized by grotesque deformities and are usually irreversible.
1.6 Development of the disease

This may be classified into the following stages:

1.6.1 The pre-patent period (biological incubation period)

It is the time between entry of infection stage larvae to the development of adult worms and appearance of mf in the circulating blood of the host. It has been estimated to require a year or more. However, the *B. malayi* takes 3 - 4 months to develop in the definitive host.

1.6.2 The patent (symptom-less) period

This stage is characterized by the presence of mf in the peripheral blood but without any clinical manifestations of filariasis. This is the most important group that serves as the secondary carrier of infection. A considerable proportion of population remain microfilaraemic and asymptomatic for years together and in some instances for whole life. However some individuals become amicrofilaraemic while other may progress more rapidly to the acute and chronic stages. Most of the asymptomatic cases have lymphatic abnormalities as detected by lymphoscintigraphy and also renal abnormalities, which is evidenced by hematuria and/or proteinuria (Dreyer et al., 1992; Freedman et al., 1999).

1.6.3 The acute or allergic stage

The acute clinical manifestations of filariasis are characterized by episodic attacks of adenolymphangitis (ADL) with constitutional symptoms like fever, chills, malaise, nausea, headache and vomiting (WHO, 1998b). In bancroftian filariasis the ADL attacks occur usually in the limbs and groin while the male genitalia are the most often affected during the acute stage, leading to funiculitis, epididymitis or orchitis. The repeated attacks of ADL precede the development of chronic lymphatic pathology of filariasis and these often continue for many years (Pani et al., 1994). ADL lasts usually for 3 - 5 days but sometimes may stay up to 15 days. Lymphoedema is frequently present during the episodes and usually subsides after acute stage. However sometimes it does not subside and lead to chronic changes (Dissanayake et al., 1984).
1.6.4 The chronic manifestations

The most conspicuous feature of clinical symptoms caused as a result of filarial infection is noted in the chronic stage. This occurs due to blockage of lymphatics. The major chronic signs are hydrocoele, chyluria, lymphoedema and elephantiasis, which may differ in occurrence from one area to another. More serious are the blockage of the abdominal or thoracic lymph vessels, which eventually cause chyluria or hematochyluria. This stage is often incurable.

1.7 Categories of filarial subjects in filaria endemic area

In an endemic population almost all individuals are exposed to the mosquito bites, and so an equal probability prevails for individuals to be exposed to inoculation of L\textsubscript{3}. However all individuals do not develop into similar state of infection. Following infection with L\textsubscript{3} there is usually a period of vigorous immune responses to the invading larvae. If the larvae are not cleared from the body during this period then various pathologies associated with filarial infection can develop. Irrespective of the infection exposure dose and course of development of infection, different individuals respond differently. This is an important feature of human lymphatic filariasis that, not all hosts develop microfilaraemia (Lawrence & Devaney, 2001). So there exist groups of individuals ranging from mf negative, to infection positive but without symptoms, and to manifestation of chronic disease in the form of elephantiasis.

1.7.1 Mf carriers

Most of the people living in endemic area show mf in their peripheral blood and remain as such throughout life. These are called as mf carriers. They lack recognizable clinical manifestations in their entire life. Asymptomatic mf carriers do not always present with overt clinical manifestations, but lymphatic pathology in the form of dilation, kinking, collateral formation, etc., are common. They are involved in spreading of disease.

1.7.2 Symptomatic

These are the individuals in whom the disease has proceeded to the acute stage. The most pronounced of these is the damage to the lymphatic vessels, which is mediated by the immune responses to the adult worms living in them. These acute immune responses are characterized by Lymphangitis (inflammation of the affected area), generalized malaise and fever. Later the major chronic signs appear as hydrocoele, chyluria, lymphoedema and
elephantiasis. More serious are the blockage of the abdominal or thoracic lymph vessels, which eventually cause chyluria or hematochyluria and is often incurable. Symptoms due *B. malayi* infection are largely same as that due to *W. bancrofti*. However fever associated with lymphangitis in patients from endemic areas of *B. malayi* is more common than in those of *W. bancrofti*. Lymphoedema and elephantiasis of legs and arms are common in both but the absence of involvement of the genito-urinary organs is a characteristic feature of *B. malayi* filariasis. Though mostly limbs are affected, the upper extremities are much less frequently affected than the legs.

### 1.7.3 Endemic normal

In most of the endemic areas a proportion of the population remained mf negative and is devoid of symptoms of the disease despite life long exposure to infection. It is possibly heterogeneous group consisting of “Truly Immune” cases apart from those with pre-patent or sub clinical or unisexual infections of parasite.

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Fig. 2.4: Spectrum of human lymphatic filarial disease (Source: http://maizels group .biology. ed.ac.uk)
1.7.4 Other forms of filariasis

➤ **Occult filariasis**

The term occult filariasis is commonly used to designate filarial infections in which classical clinical manifestations are not present and mf are found at very low density in the peripheral blood although they may be seen in tissues. Occult filariasis is believed to result from a hypersensitivity reaction to filarial antigens derived from mf. Only a very small proportion of individuals in a community where filariasis is endemic develop occult forms of the disease. Occult filariasis with adult *W. bancrofti* in the anterior chamber of the eye has also been reported (Arora & Das, 1990; Nanavaty, 2001; Rao, 2008).

➤ **Tropical Pulmonary Eosinophilia**

Tropical pulmonary eosinophilia (TPE) is an occult manifestation of filariasis (Narayanan *et al.*, 2003). Its main clinical manifestations are: nocturnal paroxysms of asthmatic symptoms (severe cough and wheezing, especially at night), frequent weight loss and fatigue but with minimal or no fever, restrictive or obstructive lung abnormalities, abnormal chest radiographs that frequently show diffuse mottled pulmonary interstitial infiltrate. The peripheral blood eosinophilia increases to very high extent (> 3000 cell/µl), extreme elevation of antifilarial immunoglobulins (IgE and IgG). Dramatic clinical improvement in response to specific anti-filarial chemotherapy with (DEC) has been observed (McCarthy *et al.*, 1995).

➤ **Onchocerciasis**

Onchocerciasis (river blindness) results from infection with *Onchocerca volvulus*. In endemic regions, it is a major cause of severe disfiguring skin changes and damaging eye lesions. Dermal changes occur when the mf undergo destruction in the skin and vary from a few papules to the extensive pigmentary and chronic atrophic changes (Murdoch *et al.*, 2002). The more chronic changes are probably related to the repeated occurrence of local pathology around dying parasites. There is skin atrophy with loss of elasticity, giving a prematurely aged appearance (presbyderma). Onchocerciasis has been associated with weight loss and musculoskeletal pain, Nodule formation, eye lesion. Several reports have indicated a higher than normal frequency of epilepsy in onchocerciasis hyperendemic areas (Marin *et al.*, 2006). DEC is no longer recommended for the treatment of onchocerciasis. It can induce severe adverse reactions, especially in heavily infected individuals, and may precipitate or aggravate...
ocular lesions. It has now been replaced by ivermectin (Mectizan) as the drug of choice (Boatin & Richards, 2006). Ivermectin in a single oral dose of 150 mg/kg body weight causes a rapid elimination of microfilariae from the skin (Awadzi et al., 1989).

➢ Acute dermatolymphangioadenitis (ADLA)

A major risk factor for development of chronic lymphedema or elephantiasis is thought to be recurrent episodes of acute dermatolymphangioadenitis (ADLA). It is characterized by diffuse inflammation and swelling and occasionally associated with an ascending lymphangitis and adenitis. A distal skin lesion, often an interdigital infection, frequently serves as the entry point for bacteria precipitating ADLA (Ananthakrishnan & Das, 2004). Frequent ADLA episodes contribute to the progression of lymphedema by repeated damage to superficial lymphatic vessels (Suma et al., 1997).

➢ Acute adeno-lymphangitis (ADL)

This is the most common acute presentation. It is characterised by i) fever and painful lymphadenopathy in the groin and axillae, ii) Affected areas being painful, tender, red and swollen - usually the result of superimposed bacterial infection and iii) Occurrence several times in a year and more so in rainy seasons, when the moisture between toes increases, leading to fungal infections which damage the skin, allowing worms to invade. Each episode of ADL enhances the development of lymphoedema.

Other manifestation such as mono/polyarthritis, hematuria, glomerulopathies (glomerulonephritis), endomyocardial fibrosis, oligoarticular filarial arthritis, and filarial pseudo-rheumatism are also well recognized.

1.8 Pathogenesis

Host immune and inflammatory responses to infections that are poorly controlled may underlie various chronic diseases. Immune responses, both adaptive and innate, can lead to the development of inflammation. These responses may result in the production of antibodies of specific isotype, immune complex deposition, antibodies against cellular targets, or T cell-mediated responses. Inflammatory responses typically help to eliminate offending parasites. But those same inflammatory responses may also damage surrounding healthy tissues. If the microbe persists or if the normal signaling pathways that down-regulate the inflammatory
response are disordered, then an ongoing inflammatory response may develop. The tissue damage resulting from this response may be the dominant portion of the clinical disease (Pincus, 2005).

The pathology of lymphatic filariasis has three components: (1) parasitological (including elements of rate of infection and parasite mortality); (2) immunological (according to selection of immune mechanisms and target antigens); and (3) microbiological (secondary opportunistic infections that exploit damage induced by the presence of filariae). Dissection of these factors is complicated, especially as any group of human patients will include persons at different stages of disease development, varying from those who still retain large parasite numbers to those whose lesions have outlived the filarial worm population. Moreover, it is possible that elephantiasis can result from either parasitological or immunological causes, and the search for a unifying character may be fruitless. The parasitological component of pathogenesis includes direct effects such as lymphatic dilation induced by adult worms which reduces flow and leads to incompetence. There may also be dynamic effects related to the pattern of infection; cats infected repeatedly with small doses show a higher rate of lymphatic pathology than do those given single, large inoculums. Immunologically, elephantiasis cases show high T-cell proliferative responses and high IgE and IgG\textsubscript{1}, IgG\textsubscript{2} and IgG\textsubscript{3} to filarial antigens. However, a significant minority of these patients have a microfilaraemic-like phenotype, bearing hyporesponsive T cells with high IgG\textsubscript{4}. This finding casts doubt on either IgE or inflammatory T cells being the initiating factor in lymphatic lesions. Furthermore, many healthy endemic normals can be found with IgE and T-cell responses comparable to those seen in elephantiasis. One suggestion has been that normals avoid pathology by killing incoming parasites promptly before entry into the lymphatics. A more-interesting lead is the high levels of IgG\textsubscript{1}, IgG\textsubscript{2} and IgG\textsubscript{3} seen in elephantiasis relative to both microfilaraemics and normals; it should also be noted that these isotypes are elevated in elephantiasis patients irrespective of their T-cell or IgE responses (Maizels et al., 1995).
1.9 Disease management

1.9.1 Diagnosis

The success of any control programme depends on the sensitive diagnostic techniques. Identification of all true positive individuals in an endemic community can be problematic since filariasis is spectral and no single diagnostic technique can be expected to be uniformly sensitive in all situations. Therefore definite diagnosis of filariasis can be achieved by taking the support of evidence like history of patients having resided in filaria endemic area, presence of mf positive individual in the same home and demonstration of filarial antibodies or antigens etc (WHO, 1998a). However, the diagnosis of occult filariasis is much more difficult as the individuals hardly show mf in the circulation or classical manifestations. A favorable response to DEC provides additional evidence. Besides, supportive evidences like detection of parasite antigen or antibodies are very much helpful to establish associations of these conditions with filariasis.

LF was diagnosed clinically by blood films taken at night, as most forms of human filarial parasites have nocturnal periodicity. The limited sensitivity of blood films led to the development of concentration techniques (nucleopore filtration) or detection in larger quantities of lysed blood using a counting chamber. A DEC-based provocative test was also used in some settings if night blood films could not be done, as the treatment with DEC ‘provokes’ the appearance of microfilaria in the blood within 30-45 min of DEC administration, during the day (Molyneux, 2009).

It can also be established following a laboratory examination revealing hypereosinophilia corresponding to the incidental finding of mf (blood or skin). The visualization of the embryonic and/or adult parasite confirms the infection. For pathogenic filariasis with microfilaraemia, paradoxically, clinically positive subjects are often amicrofilaraemic. In this case, the presence of antibodies and/or specific serum antigens confirms the diagnosis (Carme, 2007). Other methods include detection of ‘filaria dance sign’ (Amaral et al., 1994).
1.9.1.1 New advances in diagnosis

The recent developments in the diagnosis of lymphatic filariasis are given below. There are three main approaches in the diagnosis of filariasis Clinical, Parasite demonstration and Immunological (detection of antibodies and parasite antigen). Among the parasitological techniques, the membrane filtration method is the most reliable (Table 2.4).

➤ **Membrane filtration method for microfilaria detection**

Venous blood drawn at night and filtered through milipore membrane filters, enables an easy detection of microfilaria and quantifies the load of infection. They are usually observed in the early stages of the disease before clinical manifestations develop. Once lymphoedema develops microfilaria are generally absent in the peripheral-blood (McCarthy, 2000).

➤ **Ultrasonography**

Recently, ultrasonography has helped to locate and visualise the movements of living adult filarial worms of *W. bancrofti* in the scrotal lymphatics of asymptomatic males with microfilaraemia. The constant thrasing movement of the adult worms in their ‘nests’ in the scrotal lymphatics is described as the ‘filaria dance sign’ (Amaral *et al.*, 1994). The lymphatic vessels lodging the parasite are dilated and this dilation is not seen to revert to normal even after the worms are killed by diethylcarbamazine administration. Ultrasonography is not useful in patients with filarial lymphoedema because living adult worms are generally not present at this stage of the disease. Similarly ultrasonography has not helped in locating the adult worms of *B. malayi* in the scrotal lymphatics since they do not involve the genitalia (Shenoy *et al.*, 2000b).

➤ **Lymphoscintigraphy**

After injecting radiolabelled albumin or dextran in the web space of the toes, the structural changes are imaged using a gamma camera. Lymphatic dilatation, dermal back flow and obstruction can be directly demonstrated in the oedematous limbs by this method. Lymphoscintigraphy has shown that even in the early, clinically asymptomatic stage of the disease, lymphatic abnormalities in the affected limbs of people harboring mf may occur (McCarthy, 2000).
Review of Literature

➢ Immunochromatographic test (ICT)

Highly sensitive and specific filarial antigen detection assays, both as card test and in ELISA based format are now available for the diagnosis of *W. bancrofti* infection. This test is positive in early stages of the disease when the adult worms are alive and becomes negative once they are dead (Weil et al., 1997). In south India, filarial diagnostic test used for field trial for assessment of infection, ICT card test gave 100% specificity and 98.5% sensitivity over finger prick and membrane filter method for determining mf in peripheral blood in endemic and none endemic area of *W. bancrofti* (Table 2.5) (Pani et al., 2000).

➢ DNA probes using Polymerase Chain Reaction (PCR)

These tests are of high specificity and sensitivity, and are able to detect parasite DNA in humans as well as vectors in both bancroftian and brugian filariasis (Walther & Muller, 2003).

Table 2.4: Diagnostic tests: Parasitological.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test</th>
<th>Merit</th>
<th>Demerit</th>
<th>Acceptability</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Counting chamber (20-100 cmm blood)</td>
<td>Relatively quick</td>
<td>Poor sensitivity Night blood sampling difficult Permanent preparation Speciation difficult</td>
<td>Not fit for field use</td>
<td>(Desowitz, 1971)</td>
</tr>
<tr>
<td>ii</td>
<td>Membrane filtration (1-5 ml blood)</td>
<td>Very sensitive</td>
<td>Expensive Night blood sampling difficult</td>
<td>Not fit for field use Can be used selected cases</td>
<td>(Shibuya et al., 1977)</td>
</tr>
<tr>
<td>iii</td>
<td>DEC provocation</td>
<td>Can be performed in day time</td>
<td>Poor sensitivity</td>
<td>Not fit for mass survey Can be used in selected cases</td>
<td>(Murthy et al., 1983)</td>
</tr>
<tr>
<td>iv</td>
<td>Thick Blood smear (20-60 cmm blood)</td>
<td>Less expensive</td>
<td>Poor sensitivity Night blood sampling</td>
<td>Acceptable for surveys</td>
<td>(Chandra et al., 1986)</td>
</tr>
<tr>
<td>v</td>
<td>DNA based diagnostic tests</td>
<td>Highly sensitive</td>
<td>Cross reaction</td>
<td>Very suitable for field use</td>
<td>(Walther &amp; Muller, 2003)</td>
</tr>
</tbody>
</table>
Table 2.5: Diagnostic tests: Immunological.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test</th>
<th>Merit</th>
<th>Demerit</th>
<th>Acceptability</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.</td>
<td>Antibody assay</td>
<td>Suitable where diagnosis is needed to commence treatment</td>
<td>Cannot differentiate between present and past infection, Occasional false negativity due to immunosuppression</td>
<td>Fit for measuring exposure rate also</td>
<td>(Kagan &amp; Norman, 1963)</td>
</tr>
<tr>
<td>ii.</td>
<td>Gel-diffusion</td>
<td>Less expensive</td>
<td>Least sensitivity</td>
<td>Unfit for field use</td>
<td>(Kagan &amp; Norman, 1963)</td>
</tr>
<tr>
<td>iii.</td>
<td>Counter current immunoelectro-phoresis</td>
<td>Relatively quick</td>
<td>Low sensitivity</td>
<td>Unfit for field use</td>
<td>(Kagan &amp; Norman, 1963)</td>
</tr>
<tr>
<td>iv.</td>
<td>Indirect fluorescent antibody</td>
<td>Medium sensitivity</td>
<td>Expensive, Requires sophisticated instrument and expertise</td>
<td>Unfit for field use</td>
<td>(Pillot et al., 1976)</td>
</tr>
<tr>
<td>v.</td>
<td>Immediate hypersensitivity</td>
<td>Highly sensitive, Easy to perform, Quick and Results are on the spot</td>
<td>Best results with homologous antigens</td>
<td>Very suitable for field use</td>
<td>(Grove et al., 1977)</td>
</tr>
<tr>
<td>vi.</td>
<td>Indirect haemagglutination</td>
<td>Medium sensitivity</td>
<td>Cross reaction, Time consuming</td>
<td>Unfit for field use</td>
<td>(Kharat et al., 1981)</td>
</tr>
<tr>
<td>vii.</td>
<td>Antigen assay</td>
<td>Unequivocal, Indicate current infection</td>
<td>Poor sensitivity, Cross reactivity with other parasites, Needs purified antigens, less sensitive for early and light infection</td>
<td>Moderately suitable for field use</td>
<td>(Hamilton et al., 1983; Karavodin &amp; Ash, 1980)</td>
</tr>
<tr>
<td>viii.</td>
<td>Enzyme linked immunosorbent assay</td>
<td>Highly sensitive</td>
<td>Expensive, Time consuming, Requires sophisticated facilities, and expertise Need purified antigens</td>
<td></td>
<td>(Tandon et al., 1988)</td>
</tr>
</tbody>
</table>
1.9.2 Treatment and control

I. Palliative treatment

Pathological symptoms may be treated at an early stage. There is no drug which can reduce grotesque swelling. Report is available that DEC with Coumarin can reduce pathological swelling up to some extent. Repeated cleaning of the affected portion with soap and water and application of antibiotic-antifungal creams have a dramatic effect on the elephantoid limbs. Raising the affected limb and excising limb to promote lymph flow. Keeping nail clean and wear shoes. Using local antiseptic or antibiotic creams to treat small wounds or abrasions are the Palliative treatment. Asymptomatic should be treated to stop aggravation of the disease symptoms as they have hidden lymphatic damages. Other symptoms like TPE, chyluria are treated with DEC as no other drug is available. Finally surgical treatment needed.

II. Diethylcarbamazine (DEC)

This drug is effective against both mf and adult worms. DEC markedly lowers the blood mf levels even in single annual doses of 6 mg/kg, and this effect is sustained even after one year. Even though DEC kills the adult worms, this effect is only observed in 50% of patients. This drug does not act directly on the parasite but its action is mediated through the immune system of the host. The sustained destruction of mf by this drug even in annual single doses makes it a good tool to prevent the transmission of this disease. The adverse effects produced by the drug are mostly observed in patients who have mf in their blood and are due to their rapid destruction which is characterized by fever, headache, myalgia, sore throat or cough lasting for 24 to 48 hr (Andrade et al., 1995). They are usually mild and self-limiting requiring only symptomatic treatment. DEC is the drug of choice in the treatment of Tropical Eosinophilia syndrome in which it should be given for longer periods of 3 to 4 weeks.

III. Ivermectin (IVM)

This drug acts directly on the mf and in single doses of 200 to 400 µg/kg keeps the blood mf counts at very low levels even after one year, such as DEC. The adverse effects noticed in microfilaraemic patients are similar to those produced by DEC but are milder due to the slower clearance of the parasitemia. IVM has no proven action against the adult parasite or in tropical eosinophilia (Dreyer et al., 1996). IVM is the drug of choice for the treatment of
onchocerciasis because of its safety and efficacy, when compared to DEC. It is also the drug of choice for the prevention of filariasis in African countries endemic for Onchocerca and Loa loa, where DEC cannot be used due to possible severe adverse reactions.

IV. Albendazole (ALB)

This antihelmintic drug is shown to destroy the adult filarial worms when given in doses of 400 mg twice a day for two weeks. The death of the adult worm induces severe scrotal reactions in bancroftian filariasis since this is the common site where they are lodged (Jayakody et al., 1993). ALB has no direct action against the mf and does not immediately lower the mf counts. When given in single dose of 400 mg in association with DEC or IVM, the destruction of mf by these drugs becomes more pronounced. ALB combined with DEC or Ivermectin is recommended in the global filariasis elimination programme.

IV. Combination therapy

The above drugs have also been studied for possible synergistic effects by co-administration of the standard drugs like ALB + IVM, ALB+DEC, ALB+DEC+IVM, or DEC+IVM in various population. The strategy that appears most suitable for the elimination of filariasis in India is the administration of a single annual dose of ALB 400 mg along with DEC 6 mg/kg of body weight (Table 2.6). This not only prevents transmission of filariasis in the community by reducing the mf levels, but also has the added benefit of clearing the intestinal helminths (Shenoy et al., 2000a). Results with ALB added to single-drug therapy with IVM or DEC against lymphatic filariasis were inconclusive, but DEC and IVM in combination appeared to be superior to DEC or IVM alone. None of the drug combinations against lymphatic filariasis showed more adverse reactions than single-drug therapy (Olsen, 2007).

Table 2.6: WHO described drug usage for Mass Drug Administration in LF endemic areas

<table>
<thead>
<tr>
<th>Disease prevalent</th>
<th>Drugs</th>
<th>Dosages</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF in countries co-endemic for onchocerciasis in Africa</td>
<td>Albendazole + Ivermectin</td>
<td>Albendazole: one tablet per person (&gt;90 cm in height), Ivermectin: use of an Ivermectin dose pole or 200-400 µg/kg</td>
</tr>
<tr>
<td>LF but no onchocerciasis</td>
<td>Albendazole + DEC</td>
<td>Albendazole: one tablet per person (aged &gt;2 years), DEC: 6mg/kg body weight</td>
</tr>
</tbody>
</table>
The antifilarial agents used till now with their mode of action and adverse effects are briefed in Table 2.7.

**Table 2.7: Effective antifilarial agents**

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Agent</th>
<th>Use</th>
<th>Mode of action</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperazine</td>
<td>Diethylcarbamazine</td>
<td>MIF</td>
<td>Hyper-polarization of Muscle Membrane (Del Castillo et al., 1964)</td>
<td>Adverse systemic reaction can be reduced by prolonged treatment of multiple low doses Nephrotoxic</td>
</tr>
<tr>
<td>Cyanine Dyes</td>
<td>Cyanine-863</td>
<td>MAF</td>
<td>Inhibition of Glucose Transport (Mansour et al., 1960)</td>
<td>Toxic</td>
</tr>
<tr>
<td>Arsenicals</td>
<td>Arsenamide</td>
<td>MAF</td>
<td>Binds to Sulphhydryl proteins (Otto et al., 1952)</td>
<td>Toxic</td>
</tr>
<tr>
<td>Imidazole</td>
<td>Levamisole</td>
<td>MAF/MIF</td>
<td>Cholinergic agonist (Forbes, 1972)</td>
<td>Neurotoxic</td>
</tr>
<tr>
<td>Organo-Phosphate</td>
<td>Metrifonate</td>
<td>MAF</td>
<td>Inhibition of Acetyl cholinesterase (Bueding et al., 1972)</td>
<td>Toxic</td>
</tr>
<tr>
<td>Naphthalene Sulphonic acid</td>
<td>Suramin</td>
<td>MAF</td>
<td>Inhibition of folate Metabolism (Jaffe et al., 1980)</td>
<td>Nephrotoxic</td>
</tr>
<tr>
<td>Amosconate</td>
<td>Isothiocyanate</td>
<td>MIF/MAF</td>
<td>Inhibitory effect on Carbohydrate Metabolism (Nelson &amp; Saz, 1984)</td>
<td>Hepatotoxic</td>
</tr>
<tr>
<td>Antimonial</td>
<td>Stibophen</td>
<td>MAF</td>
<td>Inhibition of Phosphofructokinase (Saz &amp; Dunbar, 1975)</td>
<td>Toxic</td>
</tr>
<tr>
<td>Nitro furans</td>
<td>Furazolidon</td>
<td>MAF</td>
<td>Inhibition of phosphorylphosphatase (Bueding &amp; Fisher, 1970)</td>
<td>Fever and irritation of digestive tract</td>
</tr>
<tr>
<td>Avermectin</td>
<td>Ivermectin</td>
<td>MIF</td>
<td>Chloride Channel opener at GABA mediated interneuron (Duce &amp; Scott, 1985)</td>
<td>Adult worm persist</td>
</tr>
<tr>
<td>Benzimidazole</td>
<td>Albendazole</td>
<td>MAF</td>
<td>Interruption of Microtubular function (Lacey &amp; Prichard, 1986)</td>
<td>Poor gastrointestinal absorption, Teratogenicity</td>
</tr>
</tbody>
</table>

MAF – Macrofilaricidal  MIF – Microfilaricidal
Data reported to WHO by the end of July 2011 showed that during 2010 MDA targeted 622 million people, and 466 million were treated; thus, the reported coverage was 75%. In addition to data for 2009 reported in 2010, 2 countries – India and Nigeria – submitted their final reports for 2009. In 2009, the total number of people treated by MDA was 485 million, about 100 million more than reported in 2010. All of the 5 WHO Regions with affected countries, except the South-East Asia Region and the Western Pacific Region’s Mekong-Plus Programme Review Group, treated more people in 2010 than in 2009.

**Fig. 2.5:** Countries where lymphatic filariasis was endemic in 2010 and the status of MDA in those countries (WHO, 2012).

### 1.9.3 Global Programme to Eliminate Lymphatic Filariasis (GPELF)

The Global Programme to Eliminate Lymphatic Filariasis (GPELF) targets the global elimination of lymphatic filariasis as a public-health problem by 2020. The programme recommends a comprehensive strategy for achieving the elimination goal through a two-pillar approach: (i) interruption of transmission of filarial infection in all endemic countries through
mass drug administration (MDA); and (ii) prevention and alleviation of disability and suffering in individuals already affected by LF.

GPELF is one of the most rapidly expanding public-health programmes in the world. Of the 73 countries where LF is endemic, 53 countries have started implementation of MDA, of which 12 countries have implemented more than 5 rounds of MDA and transitioned to post-MDA surveillance.1 When GPELF reached its half-way point in 2010, the World Health Organization (WHO) reviewed the progress made during 2000–2009 and developed a strategic plan to address the challenges in the next 10 years.2 Since then, GPELF has progressed towards the targets and milestones set in the Strategic Plan, and supported endemic countries to start and scale up MDA, phase into post-MDA surveillance and achieve verification of elimination. (Strategic and Technical Advisory Group for neglected tropical diseases Report of a meeting of the subworking group on monitoring and evaluation of disease-specific indicators – lymphatic filariasis Task Force for Global Health, Atlanta, GA. October 2012).

Table 2.8: Estimated impacts of the Global Programme to Eliminate Lymphatic Filariasis, 2000–2008a.

<table>
<thead>
<tr>
<th>Impact on health</th>
<th>No. of people protected (million)</th>
<th>Cost savings (billion US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention of infections in newborns</td>
<td>8.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Prevention of progression from subclinical to clinical disease</td>
<td>10.7</td>
<td>16.5</td>
</tr>
<tr>
<td>Prevention of worsening morbidity or reversal</td>
<td>2.2</td>
<td>5.4</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>24.2</td>
</tr>
</tbody>
</table>

Source: The economic benefits resulting from the first 8 years of the Global Programme to Eliminate Lymphatic Filariasis (2000–2007)
1.10 Vector control

It is the primary tool for controlling filariasis in several part of world and also prevents other diseases simultaneously (Burkot et al., 2007). Covering water-storage containers and improving waste-water and solid-waste treatment systems can help by reducing the amount of standing water in which mosquitoes can lay eggs. In addition, killing eggs (ovicidal) and killing or disrupting larva (larvicidal) in bodies of stagnant water can further reduce mosquito populations.

I. Foot-care programme

Some recent studies have shown that with proper ‘local care’ of the affected limb these ADL attacks can be prevented even in case of severe lymphoedema. This ‘foot-care programme’ involves washing of the affected area, especially the webs of the toes and deep folds of skin, with soap and water twice a day or at least once before going to bed and wiping dry with a clean cloth to avoid moisture; clipping the nails at intervals and keeping them clean; preventing or promptly treating any local injuries or infections using antibiotic ointments (McCarthy, 2000). Regular use of properly fitting foot wear; raising the affected limb at night in order to reduce the swelling; to prevent repeated ADLs in such patients, administration of long term antibiotic therapy with oral penicillin or long acting parenteral benzathine penicillin (Palumbo, 2008).

Once lymphoedema is established there is no cure. However the following treatment offers relief and may prevent further progression of the swelling: using elastocrepe bandage or tailor made stockings while ambulant; keeping the limb elevated at night or while resting, after removing the bandage; regular exercise of the affected limb; regular light massage of the limb to stimulate the lymphatics and to promote flow of lymph towards larger patent vessels; intermittent pneumatic compression of the affected limb using single or multicell jackets; heat therapy either using wet heat or hot oven; surgical procedures such as lymph nodo-venous shunts, omentoplasty, excisional surgery, skin grafting; prolonged treatment with oral or topical coumarin or flavonoids is considered to be effective in reducing the lymphoedema (Palumbo, 2008).
II. Antiwolbachial therapy

There is another complementary chemo-therapeutic approach that leads to a long-lasting reduction of the pathology-inducing worm stages, or to a macrofilaricidal effect (Table 2.9).

**Table 2.9: Antibiotics evaluated in antiwolbachial therapy**

<table>
<thead>
<tr>
<th>Model</th>
<th>Antibiotic</th>
<th>Dose &amp; Day</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jird- <em>B. pahangi</em></td>
<td>Tetracycline</td>
<td>1.2%; Up to 32-57 dpi</td>
<td>Inhibition of embryogenesis</td>
<td>(Bandi <em>et al.</em>, 1999)</td>
</tr>
<tr>
<td>(pre-adult stage)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. sigmodontis</em>- BALB/c mice</td>
<td>Tetracycline</td>
<td>50 mg/kg; 1-63 dpi</td>
<td>Growth retardation, infertility</td>
<td>(Hoerauf <em>et al.</em>, 1999)</td>
</tr>
<tr>
<td>(Pre-adult/adult)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. sigmodontis</em>- Cotton rat (Adult)</td>
<td>Tetracycline</td>
<td>0.3%; 150-198 dpi</td>
<td>Reduced fertility and microfilaremia</td>
<td>(Hoerauf <em>et al.</em>, 1999)</td>
</tr>
<tr>
<td><em>L. sigmodontis</em>- Multimammate rat (Pre-adult)</td>
<td>Tetracycline</td>
<td>25 mg/kg; 1-70 dpi</td>
<td>Growth retardation and infertility</td>
<td>(Hoerauf <em>et al.</em>, 1999)</td>
</tr>
<tr>
<td><em>D. immitis</em>- Dog</td>
<td>Doxycycline</td>
<td>20 mg/kg; 30 days</td>
<td>Inhibition of embryogenesis</td>
<td>(Bandi <em>et al.</em>, 1999)</td>
</tr>
<tr>
<td>(Adult)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Jird- <em>B. pahangi</em></td>
<td>Chloramphenicol</td>
<td>6.25-100 mg/kg; 15 doses during 21 days</td>
<td>No reduction in adult worms</td>
<td>(Townson <em>et al.</em>, 2000)</td>
</tr>
<tr>
<td>(Adult)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Jird- <em>B. pahangi</em></td>
<td>Oxytetracycline</td>
<td>100 mg/kg; 16 doses during 26 days</td>
<td>Animal toxicity; reduction in adult recoveries; effects on embryogenesis</td>
<td>(Townson <em>et al.</em>, 2000)</td>
</tr>
<tr>
<td>(Adult)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Jird- <em>B. pahangi</em></td>
<td>Rifampicin</td>
<td>100 mg/kg; 16 doses during 26 days</td>
<td>Animal toxicity; reduction in adult recoveries; effects on embryogenesis</td>
<td>(Townson <em>et al.</em>, 2000)</td>
</tr>
<tr>
<td>Model</td>
<td>Antibiotic</td>
<td>Dose &amp; Day</td>
<td>Effect</td>
<td>Reference</td>
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<td>------------------------</td>
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<td>--------------------------</td>
</tr>
<tr>
<td><em>O. lienalis</em>- Mice</td>
<td>Oxytetracycline</td>
<td>6.25-100 mg/kg; 15 days</td>
<td>Microfilaricidal</td>
<td>(Townson <em>et al.</em>, 2000)</td>
</tr>
<tr>
<td>(as surrogates)</td>
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<tr>
<td><em>O. lienalis</em>- Mice</td>
<td>Chloramphenicol</td>
<td>100 mg/kg; 15 days</td>
<td>Microfilaricidal</td>
<td>(Townson <em>et al.</em>, 2000)</td>
</tr>
<tr>
<td>(as surrogates)</td>
<td></td>
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<tr>
<td><em>O. lienalis</em>- Mice</td>
<td>Rifampicin</td>
<td>100 mg/kg; 15 days</td>
<td>Microfilaricidal</td>
<td>(Townson <em>et al.</em>, 2000)</td>
</tr>
<tr>
<td>(as surrogates)</td>
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<tr>
<td><em>O. lienalis</em>- cattle</td>
<td>Oxytetracycline</td>
<td>10 or 20 mg/kg; Intermittent</td>
<td>Macrofilaricidal; resolution</td>
<td>(Langworthy <em>et al.</em>, 2000)</td>
</tr>
<tr>
<td>(Adult/microfilaria)</td>
<td></td>
<td>for 6 months</td>
<td>of nodules; inhibition of</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>embryogenesis</td>
<td></td>
</tr>
<tr>
<td><em>O. volvulus</em>- Humans</td>
<td>Doxycycline</td>
<td>100 mg; 6 weeks</td>
<td>Inhibition of embryogenesis</td>
<td>(Hoerauf <em>et al.</em>, 2000)</td>
</tr>
<tr>
<td>(Adult)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O. lienalis</em>- cattle</td>
<td>Oxytetracycline</td>
<td>10mg/kg then 20mg/kg/month;</td>
<td>Microfilaricidal</td>
<td>(Bandi <em>et al.</em>, 2001)</td>
</tr>
<tr>
<td>(Adult/microfilaria)</td>
<td></td>
<td>14 days, 5 months</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The recent discovery that depletion of *Wolbachia* endosymbionts by tetracycline antibiotics leads to long-lasting sterility of adult female worms in onchocerciasis and a macrofilaricidal effect in LF fulfils these requirements. The antiwolbachial chemotherapy can currently be applied in the form of a suitable doxycycline regimen for 6 weeks for the treatment of individuals, and exploited in the future for the development of new drugs suitable for mass application. In addition, first data suggest that Wolbachia may also be major mediators of lymph angiogenesis and that their depletion is associated with reduction of lymph vessel-specific vascular endothelial growth factors and reduced lymph vessel size (Hoerauf, 2006).
III. Rapid diagnostic kit for filariasis

A rapid diagnostic kit for the detection of filariasis has been launched commercially which enables the detection of the antibodies in few minutes. The product called Signal-MF is the World's first rapid antibody detection kit for simultaneous detection of *W. bancrofti* and *B. malayi*. The current method of obtaining midnight sample of blood for diagnosis of filarial infection is not suitable for mass surveillance. Though there are imported rapid tests available for the detection of filarial infections, the factors such as high cost, stability and specificity to parasite affects their applications in developing countries endemic to the disease.

This antibody detection rapid test kit has been developed by Professor P. Kaliraj of Anna University and his team in collaboration with M/s Span Diagnostics Ltd. (ISO :9000 ;WHO-cGMP Company), Surat, Gujarat. It has passed through the stability testing as per European Pharmacopoeia and has also undergone both national and global surveillance, therefore recommended for use in India and other countries. The Human lymphatic filariasis is highly debilitating disease caused by *W. bancrofti* and *B. malayi* and affects more than 1.2 billion of the world population. More than one third of the endemic population is in India (http://dst.gov.in/whats_new/press-release07/rapid.htm).

1.11 Drug targets

Current filariasis control strategies are not entirely successful and filarial infections are on the rise. In the absence of availability of antifilarial vaccines, chemotherapy remains the mainstay for treatment of the diseases caused by filarial nematodes. There are numbers of drug targets for treatment of filaria like-

I. Carbohydrate metabolism enzymes

Carbohydrates play a significant role in providing energy to filarial species. Filarial parasites have active glycogenic and glycolytic pathways and a somewhat submissive tricarboxylic acid cycle (TCA). The enzymes involved in this pathway like Phospho-fructokinase, Lactate dehydrogenase, Fructose 1,6-bisphosphate, aldolase and other respiratory enzymes responsible for glucose uptake, transport, incorporation and utilization can be used as antifilarial drug targets (Gupta & Srivastava, 2005).
II. Trehalose metabolism enzymes

Trehalose is a sugar present in many nematode species but absent in mammals. The synthesis, accumulation and utilisation of trehalose by nematodes are important in interaction with their external environment, in osmoregulation, in resistance to desiccation, in cryopreservation and in egg-hatching. Trehalose also functions as a reserve carbohydrate fuel for energy metabolism. The combined action of two enzymes, i.e. trehalose 6-phosphate synthase and trehalose 6-phosphate phosphatase, catalyse the synthesis of trehalose in most organisms. Thus trehalose metabolism may provide new targets for attacking nematodes parasitic in mammals. In filariids, trehalose has been detected in adult *L. carinii*, *B. pahangi* and *D. viteae* and studies indicate that trehalose is synthesized from glucose (Powell *et al.*, 1986).

III. Chitin metabolism enzymes

Chitinases have been identified in three separate stages in the filarial life cycle and proposed to be a parasite unique target, as the vertebrate host does not contain chitin (Raghavan *et al.*, 1994). In adult females this enzyme plays a role in embryogenesis. However the biological role of this enzyme in mf and third stage larvae is not yet clear.

IV. Lipid metabolism: HMG-CoA reductase

The filarial isoprenoid biosynthetic pathway leads to the formation of quinones, dolichols, geranyl geraniol, juvenile hormones and purine derivatives of isopentenyl pyrophosphate (Comley, 1985). Considering the variety of important biochemical roles attributed to isoprenoids, it is possible that selective inhibition of isoprenoid biosynthesis at the HMG-CoA reductase catalysed rate limiting stage could have drastic consequences on filariae (Gupta & Srivastava, 2005).

V. Protein metabolism: cystathionine-β-synthase

Parasitic helminths are able to take up amino acids from their surroundings through their tegument. However, the identification of a novel, non-mammalian form of cystathionine-β-synthase in nematodes may facilitate the selective inhibition of a parasite-specific enzyme (Walker & Barrett, 1991).
VI. Collagen metabolism: prolyl-4-hydroxylase

The nematode cuticle consists of a network of collagen molecules, which are primarily held together by disulfide bonds. The central enzyme involved in the biosynthesis of collagen is prolyl-4-hydroxylase. This enzyme has been a subject of acute concern as a potential chemotherapeutic target, because molecules inhibiting its activity might be expected to be relatively specific inhibitors of collagen biosynthesis (Hanauske-Abel, 1991).

VII. Nucleic acid metabolism - DNA topoisomerase II

DNA topoisomerases are the enzymes required for the replication, transcription and recombination of DNA. Previously some prototype pyrimidine derivative molecules have been synthesized, which have shown adulticidal and antiinflammatory activities with additional DNA topoisomerase II inhibitory activity. These compounds possessed both macrofilaricidal and microfilaricidal actions combined with sterilizing effect due to DNA topoisomerase II inhibitory activity. In addition some pyrido-indole and quinolone derivatives (Srivastava et al., 2000) and trisubstituted pyrimidine derivatives (Katiyar et al., 2005) were also reported as novel antifilarial agents, exhibiting topo II inhibitory activity.

VIII. Folate metabolism enzymes

Folate derivatives are concerned with the transport and inter-conversion of carbon units for synthetic reactions. The high synthetic capacities of parasites and the differential sensitivities of certain folate metabolizing enzymes to inhibitors means that folate metabolism is a potential area for chemotherapy. Adult filariae possess an array of enzymes involved in the interconversion of folate analogues (Jaffe et al., 1980). DEC also inhibits a number of folate metabolism enzymes but it remains to be determined whether the ability of DEC to interfere with multiple aspects of filarial folate-related metabolism is in any way related to the antifilarial action of this drug. Suramin inhibits the dihydrofolate reductase of O. volvulus and NADP-dependent 10formyl FH4 dehydrogenase of B. pahangi (Gupta & Srivastava, 2005).

IX. Biogenic amine metabolism enzymes

Parasite-specific putrescine-N-acetyltransferase and polyamine oxidase, both involved in the reversed pathway of polyamine metabolism, were demonstrated for A. suum and O. volvulus (Müller et al., 1988). The polyamine metabolism of filaria and other helminths is still a neglected area of research, although there are reports about distribution pattern of polyamines
and some peculiarities of polyamine metabolism in filarial worms. However DFMO (DL-α-difluoromethylornithine) and MGBG (methylglyoxal bis-guanylhydrazone), both of which are potent inhibitors of polyamine synthesis in mammals, do not significantly affect the viability of filarial worms (Müller et al., 1988).

**X. Glutathione metabolism enzymes**

Glutathione (GSH) is of major importance in filarial species because it has been proposed to constitute the antioxidant system responsible for the long term existence of filarial worms in mammalian hosts by protecting them from the reactive oxygen species produced by normal metabolism and by immune cells of the host (Brophy & Pritchard, 1994). The GSH as a substrate to various enzymes like glutathione peroxidase (GPX), and glutathione-S-transferase (GST) to quench the free radicals. After GSH has been oxidized to GSSG, the enzyme glutathione reductase (GR) accomplishes the recycling of GSSG back to GSH (Lomaestro & Malone, 1995). Out of all the enzymes that are responsible for the synthesis and breakdown of GSH constitutes the γ-glutamyl cycle. At present only two enzymes of the γ-glutamyl cycle viz. glutamate-cysteine ligase (GCL) and γ-glutamyl transpeptidase (γ-GT) have been characterized from filarial species (Luersen et al., 2000). Therefore, it seems useful to develop drugs that could selectively deplete or distort glutathione stores in these parasites.

**XI. Acetylcholine receptors as targets**

The most convincing evidence that levamisole acts as a cholinergic agonist at the neuromuscular junction, comes from the study on cholinergic receptors of *C. elegans*. Further support for a cholinergic mechanism for the paralyzing action of levamisole is provided by the observation that mutants of *C. elegans*, highly resistant to the paralyzing effects of levamisole, respond very poorly to cholinergic agonists (Lewis et al., 1980). Since excitatory neuromuscular transmission in nematodes is cholinergic, acetylcholine esterase (AchE) is required for the postsynaptic inactivation of acetylcholine. Inhibition of AchE resulted in continued depolarization of postsynaptic junctions with resultant paralysis (Gupta & Srivastava, 2005).
1.12 Newer targets from *Brugia* genome

Mapping *B. malayi* genes on to the *C. elegans* protein-protein interaction network reveals an interesting pattern of evolutionarily conserved relations within the context of interconnected functional modules. In addition, defining the molecular mechanisms that allow filarial worms to persist for decades in an immunologically competent host may yield new strategies for the control of autoimmunity and the management of transplanted tissues. From the *Brugia* genome sequence data one can identify several useful systems for the discovery of additional drug targets (Ghedin et al., 2007).

**Molting:** The *B. malayi* genome contains many homologs of genes that encode molecules required for molting in *C. Elegans* (Frand et al., 2005) including proteases, protease inhibitors, nuclear hormone receptors (NRs), cuticular collagens, and chitinases.

**Nuclear receptors:** Twenty-seven members of the NR family were identified in the *B. malayi* genome including orthologs of Ecr (not present in the caenorhabditids) and other NRs acting in the *Drosophila melanogaster* ecdysone-response cascade.

**Collagens and collagen processing:** *B. malayi* has 82 genes that encode for a collagen repeat (including cuticular collagens and basement membrane collagens), which is less than half the number of collagens found in the *C. elegans* genome. It also encodes enzymes important for cuticular collagen processing such as blisterase-like proteases, protease inhibitors, tyrosinases, mixed-function oxidases, and peptidyl-prolyl isomerase.

**Neuronal signaling:** Seven putative biogenic amine heterotrimeric guanosine 5'-triphosphate–binding protein (G protein)–coupled receptors, 44 Cys-loop receptors, and 36 genes encoding potassium channels were identified in *B. malayi*, a number of which are orthologs of *C. elegans* genes that can be mutated to give paralytic or uncoordinated phenotypes.

**The *B. malayi* kinome:** The *B. malayi* genome encodes 205 conventional and 10 atypical protein kinases, of which 142 appear to be of fundamental importance based on the severity of their RNAi phenotypes in *C. elegans*.

Reliance on host and endosymbiont metabolism: As 9 of 10 enzymes required for de novo purine synthesis, 6 of 7 genes required for heme biosynthesis, and all 5 enzymes required for de novo riboflavin biosynthesis are absent from the *B. malayi* genome, the worm may be
forced to meet requirements for these key metabolic factors by active uptake of host-supplied molecules or through reliance on wBm, which has complete purine, heme, and riboflavin synthesis pathways (Foster et al., 2005).

However while having a good number of potential targets in the parasite one question that arises is - would the chemotherapeutic blockage of one enzyme alone be adequate to produce the lethal effect?

1.13 Vaccine development

The different forms of the parasite inhabit different compartments in the mammalian host. Unique set of proteins released by each form reflecting particular developmental processes and different strategies for evasion of host responses (Moreno & Geary, 2008). Other comparative analysis of proteins in secretome and adult parasite demonstrates selective release of a suite of newly identified proteins not previously suspected to be involved at the host–parasite interface, and provides important new perspectives on the biology of the filarial parasite in terms of therapeutic and vaccine targets (Hewitson et al., 2008).

Individuals living in endemic areas of filariasis require long term chemotherapy in order to prevent re-infection and transmission. However, such strategies are difficult to implement on permanent basis at the population level (Gardon et al., 1997; Molyneux & Nantulya, 2005; Tisch et al., 2005). A crucial complementary approach is the development of an effective vaccine (Volkmann et al., 2003). Although immunization with recombinant antigens has been actively investigated (Fischer et al., 2003; Wu et al., 2004; Perbandt et al., 2005), the most effective protection is still obtained with irradiated larvae. This is consistent with other helminth systems as immunization with defined antigens of Schistosoma mansoni is less effective than with attenuated infective stages (Ganley-Leal, 2005).

A variety of animal models have been used to study vaccination against filariasis and these have relevance to human pathogens because filarial parasites share many biological features including early migration through the lymphatics (Volkmann et al., 2003). Irradiated L3 vaccinations in the Litomosoides sigmodontis model to date have involved a challenge with fully infective larvae 2 weeks after their last immunization (Le Goff et al., 1997; Martin et al., 2000; Martin et al., 2001). This strategy leads to around 70% reduction in worm burden. The protective effect is established within 2 days and leads to a reduced percentage of
microfilaraemic mice 60 days post-challenge inoculation (Le Goff et al., 1997). Vaccine-induced protection is abolished when IL-5 is depleted or genetically absent, as well as in B cell deficient mice (Le Goff et al., 1997; Martin et al., 2000). In addition, degranulated eosinophils are observed at the site of challenge only in mice in which protection has occurred (Martin et al., 2001). This study suggested that antibody and eosinophils are the critical players in vaccine-mediated protection.

Irradiated L₃ larvae have been found to induce protective immunity in rodent models of filarial disease (Lucius et al., 1991; Sadanaga et al., 1984; Yates & Higashi, 1985) and several recombinant L₃ antigens have been proposed as potential vaccine candidates (Graham et al., 2001; Gregory et al., 2000). The post-infective stage and L₃ to L₄ moult are significant developmental stages and the molecules expressed at this time may have potential for vaccine development.

The immunization with *B. malayi* heavy chain myosin for example generated a high level of protection against challenge infection in jirds and *M. coucha* (Vedi et al., 2008). A multivalent vaccine formulation of *Brugia malayi* Abundant larval transcript-2 (BmALT-2) and *B. malayi* small heat shock protein (BmHSP) is an excellent vaccine for lymphatic filariasis and significant protection can be achieved against a challenge infection with *B. malayi* in a mouse model (Samykutty et al., 2010; Ziewer et al., 2012) develop a mf-based vaccine that reduces adult worm burden and prevents microfilaraemia, a powerful weapon to stop transmission of filariasis.

### 1.14 Vaccine targets

Over the past few years, a number of advancement has been made towards identifying antigens molecules for vaccine development. Earlier efforts for in search of a vaccine candidate involved use of attenuated (by irradiation) third-stage larvae (L₃) of *B. malayi* (Abraham et al., 1989) or killed mf (Hayashi et al., 1989). Later several individual proteins of *B. malayi* were also tested for their prophylactic activity and found different levels of protection. Abundant larval transcript-2 (BmALT-2), recombinant *B. malayi* transglutaminase (BmTGA), thioredoxin peroxidase (BmTPX) (Gnanasekar et al., 2004; Vanam et al., 2009) showed 35 to 70 percent activity on immunization of experimental animals. Other functional protein molecules of parasite like glutathione-S-transferase, of *Setaria cervi* (Gupta et al., 2005).
malayi Super Oxide Dismutase (BmEC-SOD) that are involved in the antioxidant system have also been exploited (Dabir et al., 2006) for such purpose. Several antigens of unknown biochemical function have also been tried showing various degrees of protection, namely - Bm-SL3 (37-kDa) (Dabir et al., 2006), Bm mf S-7 (38kDa) (Krithika et al., 2005), 175 kDa collagenase (from setaria) (Pokharel et al., 2006), filarial SXP-1 (antigen present in multiple worm stages) and r-chitinase (Wang et al., 1999).

Many other potentially protective antigens were identified basing on sero-reactivity in various population groups in endemic areas. Filarial antigens of approximately 25, 42, 60, and 112 kDa (Nilsen et al., 1988), 63 kDa antigen (Nilsen et al., 1988), 109, 102, 97 and 77; 66, 46, 35, 33, 30 and 14 kDa protein (Kazura et al., 1992; Kharat et al., 1989), 62-kDa B. malayi antigen, 120 kDa B. malayi adult antigen fraction, BmA-2 (Chenthamarakshan et al., 1995; Gaur et al., 2007) was identified and have potential to be protective in nature.

1.15 Immune response against filarial infection

Due to long association, immune response of host to parasite in filariasis infection results a wide range of clinical and pathological manifestations represented by asymptomatic carriers of mf at one pole to symptomatic cases of filariasis with or without circulating mf and extreme cases of elephantiasis to other pole.

1.15.1 Host-parasite interactions

Long association of the parasite to the immune system of host in filariasis results a wide range of clinical and pathological manifestations. To succeed in infection, parasites must have ways to reach the host, penetrate its tissues and escape its defense systems. As they are not fatal, most helminth parasites remain viable within their host for many years, exerting a strong influence over the host immune function.

1.15.2 Humoral immune responses

Increased immunoglobulin (Ig) response is well recognized in filarial infection. IgG is predominant Ig present in serum. The isotype analysis of IgG reveals that overall levels (prevalence and geometric mean intensity) of filarial-specific IgG1, IgG2, IgG4, and IgE were significantly higher in the high endemicity community than in the low endemicity community. Surprisingly, the opposite pattern was found for IgG3 (Simonsen et al., 1996; Simonsen et al.,
2002). In another study antibody subclass IgG₁, IgG₂ and IgG₃ isotypes were found to be present in very low concentration in microfilaraemic cases, but far more prominent in chronic cases (Estambale et al., 1994; Hussain et al., 1987; Ottesen, 1992). Hussain et al. (1987) observed high levels of IgG₁ in chronic patients but no difference could be detected in the IgG₂ and IgG₃ levels between the microfilaraemic and symptomatic patients. IgG₃ subclass has been reported to play some role in protection (Boyer et al., 1991), also there are known biological functions for IgG₂ apart from weak complement fixing activity and some binding to monocyte membranes (Hernandez-Pando et al., 2006). The filarial patients generate vigorous IgG₄ antibody responses to their infections (Chanteau et al., 1991; Haarbrink et al., 1995). High level of IgG₄ subclass was found in TPE cases (Ottesen, 1985) and in asymptomatic microfilaraemic individuals of lymphatic filariasis (Dreyer et al., 1992; Maizels et al., 1995; Murthy et al., 1995). IgG₄ is also known to compete with IgE for the antibody fixation sites on mast cells and eosinophils while IgE induces such cells to degranulate (Aalberse et al., 1983).

Few investigator found out that serum level of IgA is higher in chronic patients as compared to microfilaraemic patients (Chanteau et al., 1991; Hofstetter et al., 1982). IgM responses in filarial cases are still not clear; previously it was thought that IgM antibodies are related to mf clearance (Lawrence & Devaney, 2001; Weil et al., 1997). Amicrofilaraemic filarial cases have been shown to have significantly greater IgM antibody response to adult parasite antigens than the patients with microfilaraemia (Hofstetter et al., 1982; Ottesen et al., 1982). To the contrary an elevated IgM level was reported in acute filariasis while low concentrations were reported in other forms of clinical manifestations, endemic normals and non endemic normals (Ata et al., 1993). Few workers demonstrate that filaria-specific IgE production and filaria-specific IgE-mediated basophil release of histamine and IL-4 persist for years after treatment of human filarial infections. Which means parasite-specific IgE-inducing vaccines, if effective, could potentially induce longstanding protection (Mitre & Nutman, 2006). In an investigation higher IgG₄/IgE ratios were observed in infected groups than in uninfected groups. This could suggest that high levels of IgG₄ relative to IgE protect the parasite, whereas the opposite may play a role in parasite killing (Bloch et al., 2002).
1.15.3 Cellular immune responses

(Bagai & Subrahmanyam, 1970) reported first time about the involvement of cellular immunity in filariasis. They observed the death and disintegration of mf by various cells in the pleural cavity of rats infected with rodent filariid, *Limnoides carinii* which develop latency after a period of patent microfilaraemia. Later on several other investigators (Aggarwal *et al.*, 1985; Sim *et al.*, 1982) also demonstrated the antibody mediated cellular adherence and destruction of mf and L3 of various filarial species.

Delayed type hypersensitivity reactions have been assessed *in vitro* in response to filarial antigens (King & Nutman, 1992; Leiva & Lammie, 1989; Nutman *et al.*, 1987). Most of the cellular immune studies have been carried out using crude filarial antigens. Understanding the immune response at molecular levels is important for the development of strategies to cure and control of the disease. Certain fractions were identified as stimulatory (Pani *et al.*, 2000), few as suppressive (Wadee *et al.*, 1987) and some as mitogenic (Wang *et al.*, 1999). Recent research reveals the kinetics of lymphoproliferative responses of cells from renal lymph nodes that drain lymphatics harboring parasites have been shown to mimic that of the pulmonary granulomatous inflammatory response (PGRN) (Rao, 2008).

1.16 Molecular basis of immune responses

T helper 1/T helper 2 (Th1/Th2) Cell response is a classification of CD4$^+$ T cells on the basis of the patterns of cytokines that they secrete (Mosmann & Coffman, 1989). IFN-γ dominant Th1-type responses are typically evoked by microbial infections, including bacteria and viruses, and are associated with increases in the numbers of Th1 cells, cytotoxic CD8$^+$ T cells, neutrophils and macrophages. Th2-type responses are typically characterized by increase in the levels of IL-4 and other Th2-type cytokines (including IL-5, IL-9, IL-13 and IL-21), activation and expansion of CD4$^+$ Th2 cells, plasma cells secreting IgE, eosinophils, mast cells and basophils, all of which can produce several types of Th2-type cytokine (Fallon & Mangan, 2007). Although IL-10 was initially characterized as a Th2-type cytokine, recent findings show that this cytokine is also produced by Th1 cells and regulatory T cells *in vivo*, and can downregulate both Th1-type and Th2-type responses (Hoffmann *et al.*, 2000).
Several factors determine the fate of activated T cells, including antigen type, dose, type of antigen presenting cells, co-stimulatory molecules, chromatin structure, and most importantly, cytokines present in the local environment of the cell at the time of stimulation. T-box expressed in T cells (T-bet) and GATA-binding protein 3 (GATA-3) are two major T helper-specific transcription factors that regulate the expression of Th1 or Th2 cytokine genes and play a crucial role in T-helper cell differentiation. T-bet, a Th1-specific transcription factor is thought to initiate Th1 development while inhibiting Th2 cell differentiation (Shier et al., 2000). GATA-3 is a member of the GATA family of zinc finger proteins (so-called because they bind to consensus DNA sequence, A/T; GATA A/G), and plays a pivotal role in the development of the Th2 phenotype while inhibiting Th1 cells (Zhang et al., 1997). An advantage of measuring T-bet and GATA-3 expression is that this represents surrogate markers for several cells capable of producing type 1 and type 2 cytokines. FoxP3 (Forkhead box P3) is a specific marker of natural T regulatory cells (Hori et al., 2003).

Parasite antigens are presented to CD4⁺ T cells in mesenteric lymph nodes and other lymphoid tissues, driving the induction of Th2 effector cells. These cells exert their effector functions through the production of a number of cytokines, including IL-4, IL-13, IL-9 and IL-5. Th2 cells induce B-cell immunoglobulin class-switching to IgE. Innate immune cells are essential for both the initiation and effector phases of Th2-type immune responses. CD4⁺ Th2 effector cells instruct and amplify the innate effector-cell response primarily through the secretion of cytokines; once activated innate-cell populations in turn help to sustain and promote expansion of the Th2 effector-cell population. This crosstalk results in an overall effector response composed of interacting cells that coordinate and fine-tune targeted effector functions against the invading helminth parasite.

The exposure to parasites can result in three broad outcomes that are associated with specific immune responses (Fig. 2.6). One group is susceptible to infection and have immunological responses described as ‘modified T helper 2 (Th2)-cell responses. They have Th2-cell responses, with low levels of Th1 cells, and express high levels of IL-10, which might lead to tolerance. In humans, the Th2-type antibody profiles are dominated by the IgG4 isotype with relatively little IgE. These individuals often have clinically silent infections and are the main reservoir for onward transmission. At the other extreme, some develop uncontrolled inflammatory (Th1) disease. Uncontrolled inflammatory responses, often characterized by type
Ig responses in peripheral blood, are associated with lymphatic inflammation. In humans, there are only low levels of IgG\textsubscript{4} but IgE responses are evident. This leads to pathological outcomes, such as elephantiasis, which is caused by the failure of lymphatic drainage and opportunistic secondary infection. A third group seems to be resistant to infections. In this group, well-balanced immune responses would be characterized by the presence of measured Th1- and Th2-cell responses that are controlled due to the presence of regulatory activity. It is envisaged that the balanced Th1- and Th2-cell responses are of sufficient magnitude to kill the invading helminths. In humans, this is also reflected in a less skewed distribution of IgG\textsubscript{4} and IgE isotypes in the Th2-type antibody profile (Maizels & Yazdanbakhsh, 2003).

![Figure 2.6: Balance of T-cell subsets during filarial infection and their relevant outcome in the host (Maizels and Yazdanbakhsh 2003, Nature Reviews Immunology)](image_url)

To succeed in infection, parasites must have ways to reach the host, penetrate its tissues and escape its defense systems. As they are not fatal, most helminth parasites remain viable within their host for many years, exerting a strong influence over the host immune function. Many of these functions are performed by products that are released from the parasite (Moreno & Geary, 2008). Studies with mouse models confirm that, as in humans, both innate and adaptive arms of host immunity are targeted by filarial parasites (Hoerauf et al., 2005; MacDonald et al., 1998; Taylor et al., 2005).
1.17 Cells involved in inflammatory response

Macrophages

Macrophages play crucial roles in the immune response, as they can initiate, modulate and also be final effector cells during immune responses to infections. Macrophages are derived from myeloid precursor cells in bone marrow and are widely distributed in every tissue of the body. Over the past 10 years, the major support for the current concept of alternatively activated macrophages (AAMφ) or Th2 driven macrophages comes from parasitic helminth infections (Reyes & Terrazas, 2007). They become altered when a host experiences chronic exposure to helminth parasites or their by-products, which favour the induction of AAMφ. AAMφ are known to downregulate iNOS and NO production and upregulation of arginase in brugian infections in the peritoneal cavity (Allen & MacDonald, 1998). The suppressive phenotype of these macrophages is dependent on IL-4 since macrophages recruited in IL-4-deficient mice are not suppressive (Allen et al., 1996). However, infected IL-4-deficient mice do not show either increased parasite burden or pathology (Lawrence et al., 1995) suggesting that suppressive macrophages in this setting are not essential for parasite survival.
**Dendritic cells**

Innate recognition of infection in vertebrates can lead to the induction of adaptive immune responses through activation of dendritic cells (DCs). DCs are activated directly by conserved pathogen molecules and indirectly by inflammatory mediators produced by other cell types that recognize such molecules. Toll-like receptor (TLR) activation following recognition of pathogens is one of the main pathways through which DCs become activated during infections (Reis e Sousa, 2004). In patent cases of lymphatic filariasis antigen-specific T-cell unresponsiveness is observed with diminished IFN-γ and IL-2 production and defects in dendritic cell (DC) function. Mf interfere with monocyte-derived human DCs (mhDCs) function by altering TLR expression and interfering with both MyD88-dependent signaling and a pathway that ultimately diminishes NF-κB activity. This down-regulated NF-κB activity impairs DC-produced cytokines needed for full T-cell activation (Semnani et al., 2008a; Semnani et al., 2008b).

Lymphatic filariasis necessitates immune dysregulation involving APC and T cell populations. In filaria-infected individuals the baseline expression of TLR is lower in B cells than uninfected ones. Filarial Ag stimulates a diminished up-regulation of TLR in both B cells and monocytes of infected individuals (Semnani et al., 2008b). Stimulation of B cells and monocytes with TLR ligands result in decreased B cell and monocyte activation/cytokine production, indicating a state of immune tolerance. This dysregulation is associated with diminished CD4+ T cell production of IFN-γ and IL-5. The diminished expression and function of TLR is a likely consequence of chronic antigen stimulation and is one of the underlying mechanisms of dysfunctional immune response in filariasis (Babu et al., 2005).

**Activation of T cells**

T cell activation is dependent upon signals delivered through the antigen-specific T cell receptor and accessory receptors on the T cell. A primary costimulatory signal is delivered through the CD28 receptor after engagement of its ligands, B7-1 (CD80) or B7-2 (CD86). Engagement of CTLA-4 (CD152) by the same B7-1 or B7-2 ligands results in attenuation of T cells responses (Carreno & Collins, 2002). T cells from infected individuals are associated with the presence of a number of markers linked to regulatory T cells (Babu et al., 2006; Steel & Nutman, 2003). Defects in the antigen presenting cell (APC) population are also evident as
exemplified by reduced monocyte responsiveness to inflammatory stimuli (Sasisekhar et al., 2005).

❖ **Neutrophils**

Neutrophils are also activated and are recruited to sites of infection during tissue invasion by helminthes (Nair et al., 2006). Recent reports indicate an important role for neutrophils in the killing of the larval stages of *Strongyloides stercoralis*. It was shown that neutrophils were rapidly recruited to diffusion chambers containing *S. stercoralis* larvae, where they killed larvae in the absence of other cell types, although eosinophils were also required for optimal killing (Galioto et al., 2006). During infection with *S. mansoni*, neutrophils seem to have little effect, as their depletion did not influence the severity of disease. Generally, however, neutrophils are increasingly recognized as important components of the Th2-type response elicited during helminth infection. Following their rapid recruitment to sites of parasitic helminth invasion, neutrophils, working in coordination with other cell populations, including eosinophils and macrophages, can potentially directly damage tissue-dwelling helminths.

❖ **Eosinophils**

The innate immune cell populations typically associated with Th2-type responses, eosinophils, basophils and mast cells, have an integral role in antihelminth responses and in the allergic cascade (Gause et al., 2003). Following helminth infection, eosinophil numbers increase dramatically in the blood (Ganley-Leal et al., 2006) and these eosinophils rapidly migrate to the site of infection, where they degranulate, releasing eosinophil secondary granule proteins (ESGPs). Eosinophils can also have a regulatory role through the production of cytokines, including IL-4 and IL-13. However, in general, eosinophil depletion does not appear to markedly impair the development of the *in vivo* Th2-type response to most parasitic helminthes (Holland et al., 2005). A major effector function of eosinophils and their ESGPs might be in tissue remodeling and debris clearance following tissue injury, a general activity that might help to mediate the wound healing response following parasite tissue invasion (Lee & Lee, 2005).
Basophils and mast cells

Following helminth infection, basophils increase in number in the blood and tissues. Following *N. brasiliensis* infection, IL-4 producing basophils are readily detected in the lungs, liver and spleen. Basophils and eosinophils might be a major source of IL-4 at early stages of the Th2-cell response, suggesting that they promote the development of Th2 cells or their recruitment to sites of inflammation (Min *et al.*, 2004).

In lymphatic filariasis, it has been demonstrated that despite the presence of basophils and mast cells sensitized with antiparasite IgE, the allergic reactivity is controlled by blocking antibodies (Hussain *et al.*, 1992). IgE-mediated immediate hypersensitivity responses could play an important role in pathology. The IgE bound to the plasma membrane of the mediator-rich mast cells and tissue basophil cells could contribute to the inflammatory reaction, since parasite-soluble components and cuticular proteins are inducers of histamine release (Gonzalez-Munoz *et al.*, 1999; Mitre *et al.*, 2004). It is conceivable that recognition of cuticle internal matrix could occur during molting and/or after epicuticle attack by immune responses directed against other targets. If true, histamine-containing cells could participate in the response against the parasite by supporting the local inflammatory reaction. The release of products from basophils or mast cells acting on eosinophils could play a relevant role in the outcome of the infection (Pearlman, 1997).
1.18 Immune modulation: in vivo mechanisms

Fig. 2.8: Immune modulation by helminths (journal.frontiersin.org)

Active modulation of the host’s immune response is part of the parasites’ strategies for long-term survival which is characterized by a marked cellular hyporesponsiveness and a shift of the cytokine balance toward a Th2/Th3 response (Doetze et al., 2000). It is reported that the down-regulation of inflammatory reactions in the host is suggested to be induced by secreted products of the parasites (Hewitson et al., 2009) by manipulating the cytokine network, signal transduction pathways or inhibitors of essential enzymes which eventually contribute to cellular hypo-responsiveness and a possible pathogenicity factor essential for the persistence of parasite within its host. A combination of IL-10 and TGF-β produced by regulatory T cells is well known to account for immunological tolerance to cellular unresponsiveness in filarial infections (Doetze et al., 2000). CD4+ type 1 T-regulatory (Tr1) cells differentiate in the periphery from naive precursors, typically in the presence of IL-10, and ultimately regulate T-cell responses through their ability to produce IL-10 and TGF-β (Satoguina et al., 2002) (Fig. 2.8).
1.19 Role of DIM-1 protein

Cytoskeleton networks are highly ordered arrangements of structural and regulatory proteins that include actin and tubulin filaments, which make up the structural framework upon which myosin, kinesin and dyenin motor interact to perform energy dependent movement (Drubin & Hirokawa, 1988). The structure of muscle is well described and highly conserved among vertebrates and invertebrates. Striated muscle is characterized by highly ordered array of repeating structures called sarcomeres, the contractile unit of myofibrils. A sarcomere consists of interdigitating myosin thick filaments and actin thin filaments, which slide past each other producing contractile force. The sarcomere is delineated to either end by Z-disks, which are sites of attachment for thin filaments. The M-line (midline) of the sarcomere is the location of thick filaments attachment. The striated appearance of muscle results from region containing overlapping thick filaments (H-zone).

The primary sites of attachment in vertebrate skeletal muscle are located at the ends of myofibrils and contain talin, vinculin, alpha actinin, fibronectin, tenascin and integrin (Epstein & Fischman, 1991). The anchoragesitea linking the myofilaments lattice and the exoskeleton in nematodes are primarily lateral and occur at intervals along the entire length of the cell coincident with the sites of attachment for actin (Z-disk) and myosin filaments (M-line). This arrangement is reminiscent of anchorages found in vertebrate smooth and cardiac muscle cells (Francis & Waterston, 1985; Imanaka-Yoshida et al., 1999).

DIM-1 protein localizes near the basal membrane in bodywall muscle cells. The DIM-1 protein does not contain a transmembrane region so it is probably not inserted in the membrane. Most likely, it binds to one of the other proteins associated with the membrane. Interestingly, DIM-1(S) localizes just to the region around and between the dense bodies, which are the structures that anchor the actin filaments. The DIM-1 proteins contain three immunoglobulin-like repeats or domains. Ig domains have been classified into four groups, termed V, C1, C2, and I, on the basis of their sequence and their β-sheet conformation (Harpaz & Chothia, 1994; Smith & Xue, 1997). An analysis of the Ig domains of DIM-1 by the SMART analysis program (Letunic et al., 2002) reveals that the first Ig domain of DIM-1 belongs to the C2 class. The two remaining Ig domains, although similar to Ig’s in structure, do not fit into any of the four categories.
The Ig protein module is found in a diverse group of proteins including antibodies, cell adhesion molecules, cell surface receptors, and muscle proteins (Smith & Xue, 1997). DIM-1 is a member of the intracellular muscle branch of the immunoglobulin superfamily of proteins. The founding member of this branch is the Caenorhabditis elegans UNC-22/twitchin polypeptide (Benian et al., 1989) which localizes to A-bands (Moerman et al., 1988) and is thought to function in the regulation of muscle contraction (Moerman et al., 1982).

At the present time, the short DIM-1 polypeptide is found only in nematodes and is not an ortholog of any of the Ig-domain-containing muscle proteins identified in other organisms. At the protein sequence level, DIM-1 is no more similar to any of the thick filament-associated proteins than it is to any of the thin filament-associated proteins. In overall structure, DIM-1 is most similar to palladin since both proteins have only three Ig domains. However, palladin also has a unique, proline-rich, amino terminal region. Our data tentatively place the short DIM-1 polypeptide at the muscle cell membrane adjacent to the structures that anchor the thin filaments. If this subcellular localization is correct, then the primary role of DIM-1 may be in stabilizing the thin rather than the thick filament components of the sarcomere.

Recently our laboratory has shown BmAFII (Sephadex G-200 eluted fraction of adult B. malayi) with molecules ranging from 21 to 84kDa could induce host-protective immunity and conferred remarkably significant protection against B. malayi parasite challenge in M. coucha. BmAFII stimulate the release of pro-inflammatory cytokines in the order TNF-α > IL-1β = IL-6, whereas the anti-inflammatory cytokine IL-10 was barely stimulated (Dixit et al., 2004; Dixit et al., 2006). Further, 4 out of 15 SDS-PAGE fractions within the molecular weight range of BmAFII have revealed inflammation modulating molecules (Dixit et al., 2004). MALDI-TOF analysis of these 4 fractions showed 11 proteins, among which 6 proteins were identified to be immunostimulatory. Of the 4, fraction 10 (F10) induced pro-inflammatory cytokine IL-1β MALDI-TOF analysis of the fraction revealed a protein that showed extensive homology (P < 0.05) with DIM-1 of B. malayi (DIM-1bm; Acc. No.- 14972.m07771, Database search- B. malayi CDS). DIM-1bm showed similarity with As37 antigen of Ascaris suum (Sahoo et al., 2009). Tsuji et al. (2002) reported that As37 has significant similarity with C. elegans DIM-1 protein and has low similarity to part of the multi-repeat Ig domain from nematode twitchin and mammalian skeleton muscle titin, and to members of the IgSF at the amino acid sequence level. Their further study revealed that antibodies to recombinant As37 reacted with muscle cells and
hypodermis. In preliminary vaccine trials, rAs37 was found to be protective against *A. suum* in BALB/c mice. Further, the sera of mice, rabbits and pigs immunized with *A. suum* infective eggs reacted with rAs37 in immunoblot analyses (Tsuji et al., 2002) suggesting that rAs37 was immunogenic. Besides, vaccination of goats with DNA vaccine encoding DIM-1 induced partial protection against *Haemonchus contortus* (Yan et al., 2013).

To check the protective role of DIM-1 in filariasis, it is desirable to obtain this protein in adequate amount. Production of recombinant protein is the only option which further offers the possibilities of protein engineering in desired fashion. The recombinant proteins can be engineered in different ways to make it more stable and resistant for self degradation.