CLINICAL IMMUNOLOGICAL AND THERAPEUTIC ASPECTS OF LYMPHATIC FILARIASIS

Thesis submitted to

THE TAMIL NADU Dr. MGR MEDICAL UNIVERSITY

For the degree of

DOCTOR OF SCIENCE (D.Sc.,)

by

Dr. S. RAJASEKARAN, MBBS., DCP., DMRD., Ph.D.

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS
INDIAN COUNCIL OF MEDICAL RESEARCH
CHENNAI - 600031.

JULY 2016

DEDICATION

Dedicated for my Beloved, Ever Respected Teacher,

Mentor and Guide

DECLARATION BY THE CANDIDATE

I hereby declare that the post doctoral research work has been carried out independently and composed by me and has not previously formed the basis for the award of any Degree / Diploma / Associateship / Fellowship or other similar title in any other University.

Place:

Date : **SIGNATURE OF THE CANDIDATE**

CERTIFICATE

It is certified that this thesis entitled “Clinical, immunological and therapeutic aspects of Lymphatic filariasis” is a record of Post Doctoral work done by Dr.S.Rajasekaran, Research Scholar, in the department of Immunology, National Institute for Research in Tuberculosis, Chetpet, Chennai. It has not previously formed the basis for the award of any degree, Diploma, Associateship, Fellowship or any other tilte.

Dr. Srikanth Prasad Tripathy,
Scientist ‘G” and Head ,
Department of Immunology,
National Institute for Research in Tuberculosis (ICMR)
No.1. Mayor Satyamoorthy Road,
Chetpet, Chennai- 31

Dr. Sanjay Mehendale,
Director
National Institute for Research in Tuberculosis (ICMR)
No.1. Mayor Satyamoorthy Road,
Chetpet, Chennai- 31

ACKNOWLEDGEMENTS

In God We Trust

I am ever grateful to our God, the creator and guardian, and to whom I owe my very existence.

I am deeply indebted to my guide, supervisor and mentor , ever respected late **Dr.V.Kumaraswami,** MD., Ph.D., FRCP., Director-in-charge, National institute for Research in Tuberculosis, Chennai for allowing me to undertake this study and also patiently and diligently helping me in the research work; but for his guidance, help and blessings this work could not have been completed.

I am ever indebted to my teacher **Prof. Dr. K.V. Thiruvengadam**, MD., FRCP., Emeritus Professor of Medicine, Chennai Medical College, for initiating my interest in the field of Clinical, Immunological and Chemotherapeutic aspects of Lymphatic Filariasis during my training under him. His encouragement and support was instrumental for the research work on this subject, which finally led to this thesis.

I express my profound sense of gratitude to **Dr. Soumya Swaminathan,** MD., FRCP. DG ICMR, Secretary DHR, for her constant encouragement and support for the completion of this study.

I am grateful to late **Dr. R. Prabakar**, MD., Former Director, Tuberculosis Research Centre, for permitting me to undertake my Ph.D study and instrumental for D.Sc.

I am grateful to **Dr. P.R. Narayanan**, Ph.D., Former Director, Tuberculosis Research Centre, Chennai, for allowing me to continue this study and for his constant support and encouragement.

I extend my sincere thanks to **Dr. S. Geethalakshmi**, MD. Ph.D, Vice chancellor, The Tamil Nadu Dr. MGR, Medical University.

I owe my most sincere gratitude to **Dr. Sanjay Mehendale**, Director, National Institute for Research in Tuberculosis and **Dr. Srikanth Prasad Tripathy**, Head, Department of Immunology, for providing the essential support.

My sincere gratitude and heartfelt thanks for all the encouragement provided by **Dr.C.N.Paramasivam, Ph.D., Prof. Dr. V. Vijayasekaran, MD., Dr. Basheer, late Prof. SethuramanMD., Dr. P. Chandrasekar, Dr. Yesudian Patrick, Dr. K.D.Ramiah, Dr. Mohan Viswanathan, Dr. Mayil vahanan, Dr. Mohan Kameswaran, Dr. M. P. Naresh kumar, Dr. V.N. Viswanatha Rao, late Prof. Dr.S.Jeyasingh, Dr. N.D. Raghunath, Dr. V.Ravishankar, Dr. R. Ramasubramaniam, Dr. M.S. Jawahar, Dr. Rajeswari Nayak, Dr. Jeyashree Gajaraj, Dr. Geetha Haripriya, Dr.Jeyalakshmi, Dr. Kannagi, Dr. Prema David, Dr. T.S. Swaminathan, Dr. N. Kulasekaran, Dr. A. Govindan, Dr. N. Chidambaranathan, Dr. Jeyaraj, Dr. Usha Raghavan and Dr. Parthasarathy** for their constant encouragement and academic guidance.

I would like to thank **Dr. Subash Babu**, Scientific Director, International Center of Excellence, NIH –NIRT, for extending his lab facilities and support for carrying out immunological studies, for the successful completion of this work.

I would like to express my gratitude and appreciation for **Dr. G. Manokaran. MS. MCh.,** for helping me in the clinical and surgical management with follow up studies for lymphatic filariasis patients, in spite of his busy schedule.

I owe my special thanks and gratitude to **Dr. Alamelu Raja, Dr.P. Selvaraj, Dr.Sujatha Narayanan, Dr. Sulochana Das,** for their extended support and guidance during my doctoral studies.

I would like to extend my heartfelt thanks to **Dr. Ramalingam B** and **Dr. Anuradha R**, for helping me out to compile the thesis.

It has been a very pleasant experience to interact and work with **Dr. Luke Elizabeth Hanna, Dr. Uma Devi R, Dr. Vasan SK, Dr.Kripa Vasan, Dr. Kamalakkanan, Dr. Chandra kamalakannan, Dr. Pawan kumar, Ms. Shanthi**, for their company, support and affection which made my life at NIRT memorable.

My loving thanks are due to my beloved father **Thiru. C. Sivaprakasam** and my mother **Thirumathi Mrs. Nagabushnam**, my wife **R.Kalaiselvi** and my daughter **R.Priya**, my son in law **Mr.Ashok Vijayakumar** and my grandson, **Master. Shivkrish** for their everlasting love and support.

Last but not the least, I express my thanks and gratitude to all the **Patients** and **Field staff**, who had participated in this study.

Dr.S. Rajasekaran , MBBS., DCP., DMRD., Ph.D.

CONTENTS

Introduction

INTRODUCTION

Lymphatic filariasis, commonly known as elephantiasis, is a neglected tropical disease. Infection occurs when filarial parasites are transmitted to humans through mosquitoes. When a mosquito with infective stage larvae bites a person, the parasites are deposited on the person's skin from where they enter the body. The larvae then migrate to the lymphatic vessels where they develop into adult worms in the human lymphatic system. Infection is usually acquired in childhood, but the painful and profoundly disfiguring visible manifestations of the disease occur later in life. Whereas acute episodes of the disease cause temporary disability, lymphatic filariasis leads to permanent disability.

Currently, more than 1.3 billion people in 72 countries are at risk. Approximately 65 per cent of those infected live in the WHO South-East Asia Region, percent in the African Region, and the remainder in other tropical areas. Lymphatic filariasis afflicts over 25 million men with genital disease and over 15 million people with lymphoedema. Since the prevalence and intensity of infection are linked to poverty, its elimination can contribute to achieving the United Nations Millennium Development Goals.

Human lymphatic filariasis results from infection with the nematode parasites Wuchereriabancrofti, Brugiamalayi and/or B. timori. The juvenile and adult worms normally live in the lymph vessels and lymph nodes, and the microfilariae are found in the blood. The adult parasites can live for many years (probably up to 10 years, but a 40-year life-span has been reported). The life span of the microfilariae is not exactly known but may be as long as 24 months. (WHO Report)

World Health Assembly Resolution 50.29 encourages Member States to eliminate lymphatic filariasis as a public-health problem. In response, WHO launched its Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 2000. The goal of the GPELF is to eliminate lymphatic filariasis as a public-health problem by 2020. The strategy is based on two key components: interrupting transmission through annual large-scale treatment programmes, known as mass drug administration, implemented to cover the entire at-risk population; alleviating the suffering caused by lymphatic filariasis through morbidity management and disability prevention.

The Global Burden of Disease

The 2015 WHO reported on Disability Adjusted Life Years (DALYs) as a standard metric for comparing the public health impact of different diseases and conditions. The global burden of lymphatic filariasis was Mfanated at 2.8 million DALYs lost, which represents only 0.23% of the global burden of parasitic and infectious disease. However, the World Health Report estimated that lymphatic filariasis was the second most common cause of chronic disability after taking into account other factors such as the disability due to chronic disease and that due to acute attacks.

Geographical Distribution of Filariasis in Select Countries

Lymphatic filariasis is a major public health problem in tropical countries.A more recent detailed assessment of available information has attempted to correct the age, gender, and disease-specific biases in the earlier figures, and it estimates that approximately 120 million persons are infected worldwide; 107 million with *Wuchereria bancrofti* and 13 million with *Brugia malayi.* The number of people with physical disabilities due either to lymphoedema and hydrocele or the newly recognised

sub-clinical abnormalities of lymphatic and renal function are currently estimated at 43 million, with Bancroftian filariasis accounting for almost 40 million of these cases (WHO Report).

The International Task Force on disease eradication identified lymphatic filariasis as one of six potentially eradicable diseases since there are now good enough tools to combat the disease (CDC Report). The World Health Assembly at its meeting in May 1997 passed a resolution on the elimination of the disease as a public health problem through mass treatment of affected populations and appropriate management of clinical cases.

In order to initiate any disease control programme based on mass drug distribution, one needs to understand the geographical distribution of the disease in the affected countries in order to know where to target mass treatment. Unfortunately, data on the distribution of lymphatic filariasis are not widely available primarily because the standard procedures for determining which communities are affected are cumbersome, time-consuming, expensive and very intrusive. In areas where the parasite exhibits anocturnal periodicity, parasitological examinations need to be done at night. This becomes logistically cumbersome to organize, and communities often refuse to co-operate.

Recent epidemiological studies in Ghana suggested that clinical filarial disease is a good proxy measure of the levels of endemicity of filariasis. (Gyapong et al). This findings has since been validated in a WHO coordinated multi-country study (WHO). On the basis of the results, the study participants recommended the use of clinical examinations of a sample of adults as a rapid method to assess the community burden of the disease.

Even with these new rapid assessment methods, it would be very time-consuming and expensive to do filariasis surveys in all potentially endemic communities in order to determine the geographical distribution of lymphatic filariasis. However, given the clustered distribution of filariasis in most parts of the world, it may be possible to develop methods, which allow the estimation of the distribution of filariasis on the basis of surveys in a limited spatial sample of communities. Such a method has already proven very valuable for onchocerciasis control in Africa (Ngoumou et al, WHO Report).

Filarial Cripples an estimated 130 million people in the developing countries. There are according to a recent WHO estimated, 119.1 million cases of lymphatic filariasis in the world, in 76 countries and 905 million people live in areas. Where they are at risk of contacting the disease, from the biting Mosquito which transmit the filarial worms that cause serve disability and disfigurement. This amounts to106.2 million of bancroftian filariasis and 12.9 million brugian filariasis (Micheal and Bundy et al) thenumbers over physical disabilities from their infections is approximately 43 million, with bancraftian filariasis accounting for almost 40 million of these cause in the affected, the limbs well the skin hardness and stretches, producing ulcers in a mild version of the chronic stage of elephantiasis. The swelling is caused by the blockage of vessels in the lymphatic system.

The 1992 report of the WHO expert Committee on filariasis indicates that Brugian Infection is endemic in 8 Countries in South East Asian region (Bangladesh, India, Indonesia, Maldives, Myanmar, Nepal, Srilanka and Thailand) While W. Bancroft occurs in 7 countries in the American region (Brazil, Dominican republic Coasta Rica, Guyana Haiti, Suriname, Trinidadad and Tobago) 4 in the Eastern –

Mediterranean region and 17 in the western pacific region (American Samoa, Brunei, Darussalam, China, Cook Islands, Fiji, French, Polynesia, Malaysia, Nive, Papua, New Guinea, Philippines, Republic of Korea, Samoa, Tonga and Vietnam), an additional 38 Countries lie within the W.bancrofti endemic areas of sub Saharan Africa (Angola, Benin, Burkinafaso, Burundi, Cameroon Capeverde, Central Equatorial Guinea, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea Bissau, Kenya, Liberia, Madagascar, Malawi, Mali, Mauritius, Mozambique, Niger, Ramiro, Saotome and Principe, Senegal, Seychelles, Sierra Leone, Togo, Uganda, United Republic of Tanzania, Zaire, Zambia and Zimbabwe). India with 45.5 million cases and sub – Saharan with 40 million cases have very similar burdens of W. bancrofti infections. Individually, the two account for 38 percent and 34 percent, respectively of the world burden. By Comparison, hower, There are slightly higher infection and disease rates observed for the sub-Saharan than for India.

Filariasis in Asia

Asia excluding India and China is the region of third highest number of cases with 14.5 million and prevalence of 1.83 percent of bancroftian filariasis. The regional estimates for brugian filariasis suggest that India accounts for 20 percent and china about 32 percent, making up half the global burden. The largest number of cases in both the genera, W. bancroftio an B.malayi , occurs, in the 15-44 age group but the prevalence's of microfilaraemia and disease are the highest in the age group of 45 – 60 . There is also a male bias for microfilaraemia, 10 percent more in bancroftian and 25 percent more in brugian filariasis chronic disease due to bancroftian also appears to be more prevalent among males than females, largely because of the large number of hydrocoele cases put at 26.79 million. Two third of the known victims of the disease are in India, Indonesia and China, India alone, more than 300 million people are

exposed to the threat of filariasis. The efforts at controlling the disease are undermined by the increasing resistance of the parasites to drugs and the mosquito vectors to pesticides. Poor sanitation and urban squalor provide for an ideal ground for filarial mosquitoes.

Filariasis in India

To give an idea of the extent of burden of bancroftian filariasis in India, the country has 17 million cases of microfilareamia in male (with a prevalence rate of 3.87 percent), 12.46 million in females (Prevalence rate 3.04 percent). Lymphoedema cases are 2.6 million in males (0.6 percent) and 3.98 million in females (0.97 percent). Hydrocoele on the other afflicts 12.88 males (2.93 percent). These Cases amount to a total of 29.43 million males (6.7 percent) and 16.1 million females (3.92 percent). The extent of burden of being brugian filariasis in India, in the form of microfilaraemia, is1.105 million cases in males (0.25 percent), and 0.692 million in females (0.17 percent).Lymphoedema cases number 0.582 million in males (0.13 percent) and 0.282 million in females (0.07 percent). These amount to a total of 1.635 million cases in males (0.37 percent) and 0.949 million in females (0.23 percent).

In India, the National Filaria control Programme (NFCP) is a division of the National malaria Eradication Programme (NMEP) in the ministry of Health. The Primary Control Strategies of the NFCP include larviciding and environmental control measures for mosquito reduction in Urban areas, as well as screening urban Population by night blood Surveys and treating with DEC (6 mg/kg/day X 12 days) Those found either to be microfilaraemic or to have Lymphoedema. Nearly 75 percent of the population is at risk in rural areas all filariasis control efforts are confined urban areas. In India, the following states and union have been identified as endemic to filarial.

They are: Andhra Pradesh, Assam, Bihar, Goa, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharastra, Orissa, Tamil Nadu, Uttar Pradesh, West Bengal, Andaman and Nicobar Islands, Daman and Diu, Lakshadeep and Pondicherry.

Filariasis in Tamil Nadu

In the State of Tamil Nadu Sample Surveys were conducted in the mid 1970s in 11 out of 15 districts. It was found that a total of 27 million people were exposed to risk out of a total population of 41 million. One million suffered from filariasis and 1.83 million had microfilaria infections (Rao, et al). Filarias due to W.bancrofti occurred along the coastal zones of Tamil Nadu and in some inland areas. Relatively high microfilaria rates (8.3 to 11.1 percent) were also observed in the early 1955- 59 surveys under the NFCP (Sasa, et al). The estimated figures for 1985 and 1986 for infection withfilaria were 16, 425 and 18, 729 respectively. Data for rural areas were not available for the years 1985 and 1986. In the state, 13 Districts have been identified as endemic to filaria. They are Chennai(Metropolis), Kancheepuram, Thiruvallur, Vellore, Thiruvannamalai, Thiruchirapalli, Villupuram, Cuddalore, Nagapatinam, Thiruvarur, Thanjavur, Pudukottai and Kanyakumari.

The First 12 districts are under the control of the Directorate of the Public Health and preventive medicine while Chennai (until recently, Madras) under the Corporation of the metropolis. The National Filaria Control Programme (NFCP) has been, and is being implemented in Tamil Nadu, since 1957. Due to Limited financial resources, however, the fileria Disease control is at present confined to 43 urban areas only. One Survey unit is in operation at Madurai for delimiting the endemic areas in the un-surveyed district and the scheme is funded by the Central Government on 50:50 share of the cost of material and equipment.

Besides these, the state as a unique scheme for encouraging the local bodies to implement anti– filarial and anti – mosquito schemes with grant in aid from the state Government. Of the 728 local Bodies in Tamil Nadu, 174 are implementing Government approved grant in aid schemes. The public health Department has taken up some special trails for control of rural filariasis; the DEC enriched salt has been distributed in Kiliyur of Villupuram District since 1989, this trail has been very successfully, in reducing filariasis transmission as seen in the micro filarial rate of 15.12 reducing to 0.16 in 1992 and to no rate was reported in 1994.

A follow up of the successful project has been implemented in Kanniyakumari district from October 1995. The Health salt is being distributed through public distribution system (PDS) in endemic villages of this district for the control of the rural filariasis. Now, A DEC monitoring cell has been established in the year 1996. There are other programmes of control as well. A‘ single Day Mass Therapy; for example, has been conducted in august 1996 in Cuddalore district under the NFCP and DEC tablets have been distributed to 2.1 million people to avoid further spread of the disease in the area. Tamil Nadu is the first state of the Indian Union to implement the new strategy of single day mass therapy. A mass therapy has been implemented in Tanjavur and Thiruvannamalai Districts during September 1997. In Tanjavur and Nagapatinam Districts of the Cauvery Delta region in Tamil Nadu, 25 filaria and Malaria clinics have now been established (In 1997) for the diagnosis, treatment and Control of filariasis and malaria, directly under the taluk of the district headquarters Hospitals. There are in facts two district programmes in Operation in the state, namely, the NFCP and the anti – filarial scheme (AFS). The NFCP is being run in a way it takes care of mosquito collection, Dissection of mosquitoes, larvicidal activities, Night Blood Surveys, Blood

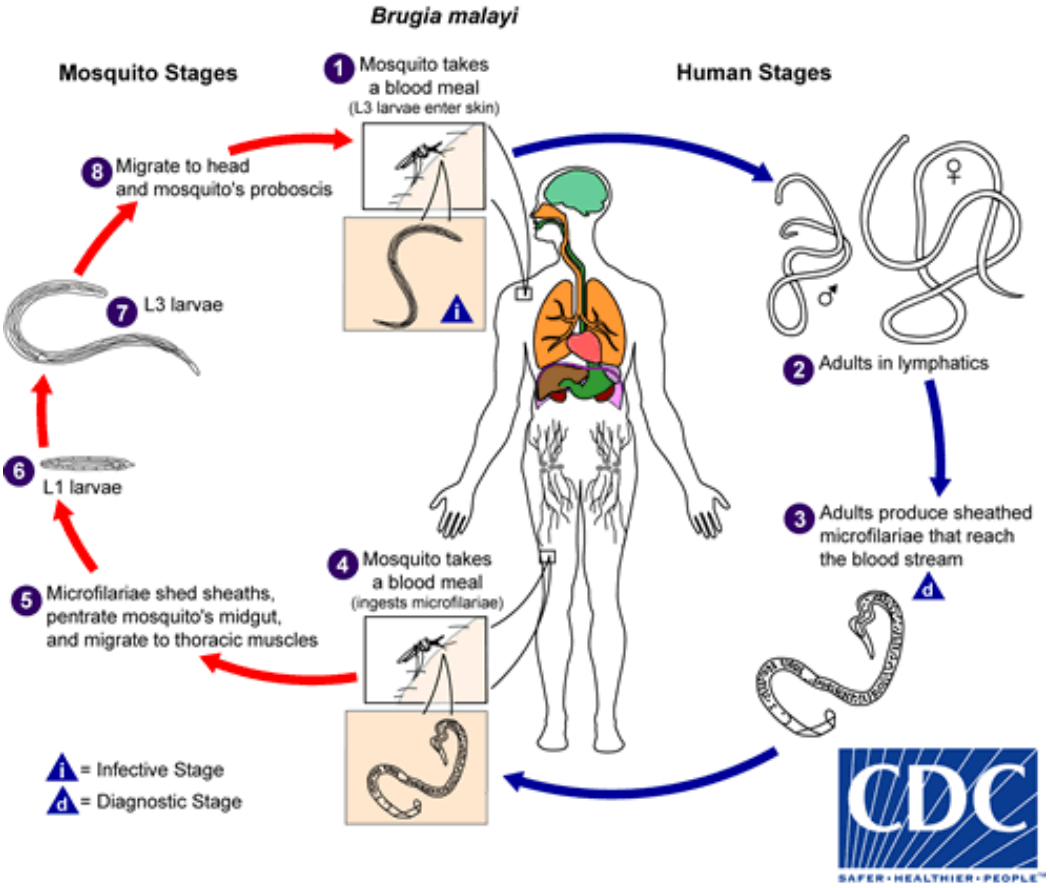
smear, Examinations, and Treatment with DEC; the AFS on the other hand, is carried out by the municipalities but with night surveys conducted by the NFCP.

Life Cycle

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

From beginning to end the duration of the *B. malayi* lifecycle is as follows: in the mosquito gut, the molt from L1 to L2 ranges from six to ten days and the molt from L2 to L3 takes one to three days. Thus, it takes approximately two weeks for the infective L3 larvae to mature in the vector. Subsequent to a bite from an infective mosquito and the entry of L3s into human skin, the L3 would transform into L4 after

nine to fourteen days in the human host, while the worm is migrating through the circulatory system to the lymphatics. The final molt, from the L4 stage to the adult worm, requires a minimum of three months but may last as long as twelve months and is localized in the lumen of dilated lymphatic vessels, the final site of the adult worm in the human body [Bain and Babayan]. *W. bancrofti*adults are typically found in the lymphatic vessels of the lower extremities in females and the lymphatic vessels of the spermatic cord in males. Although the life span of adult worms is not precisely known, it is estimated that adult females can remain reproductively active on the order of 5 years [Keiser and Nutman].



Adopted from CDC website

ELIMINATION OF LYMPHATIC FILARIASIS

During the last decade, however, simplified, safe, and cost-effective methods to control and potentially eradicate this infection have become available. For example, instead of the older 12-day treatment regimens using diethylcarbamazine (DEC), it is now clear that much simpler treatment strategies employing single yearly doses of DEC or even its daily consumption as an additive to common table/cooking salt are equally effective for control programmes and much easier and less expensive to deliver. Indeed, use of these and other techniques have already caused the elimination of lymphatic filariasis from Japan, Taiwan, South Korea and the Solomon Islands; and mainland China too is in the final stages of an exceptionally effective control programme.

Lymphatic filariasis has therefore recently been identified by the International Task Force for Disease Eradication as one of only six "eradicable" or "potentially eradicable" infectious diseases. Principally responsible for this optimism were the facts that: 1) Humans are essentially the only reservoir hosts 2) Exposure required to develop infection is high 3) Chemotherapy as the sole control method appears to be a successful strategy 4) Programmes to eradicate filariasis have already been successful. This fact, coupled with the recognition that appropriate control efforts can be effectively and inexpensively linked with pre-existing national and local public health infrastructures now provides strong impetus to initiate widespread chemotherapy programmes, with concurrent vector control where possible, aimed at finally controlling this parasitic infection and the morbidity that it causes in all endemic areas.

The specific objectives of the global programme are

a) reduction of transmission;

b) reduction of morbidity;

The levels to which the morbidity, the microfilaria rate and density, or the vector density and infectivity rate should be decreased in order for filariasis to cease to be a public health problem have not yet been agreed upon.

PARASITE BIOLOGY

Adult worms

Using ultrasound the adult worms of W. bancrofti have now been mainly localised to the scrotal lymphatic vessels in men. In women they have been found in the lymphatic tissue of the breast. They are recognised by their peculiar pattern of movement "filaria dance sign". These adult worms are found in "nests" within lymphatic vessels and remain stable. (Dreyer et al)

Life cycle of the parasites

The parasite is transmitted by mosquitoes, which serve as intermediate hosts in which microfilariae develop to the infective stage. In most endemic areas, the highest level in the human circadian or 24-hour cycle of peripheral microfilaraemia periodicity) coincides with the biting activity of the local vector. Some of the microfilariae ingested by the mosquito shed their sheaths, penetrate the stomach wall, migrate to the muscles of the thorax, and develop there without multiplication. WHO, 1984, 1992)

The slender active microfilaria transforms to the short thick inactive sausage-stage or LI larva. The LI larva has a cuticle which forms a conspicuous slender tail, characteristic of this stage. In the genus Brugia one or two nuclei are present inside the tail. After the first moult, the parasite no longer has a visible cuticle and is known as an infective or L3 larva. The L3 larva grows further in length but not in width, moving actively in the haemocoelic cavity of the mosquito, first towards the abdomen and later

to the head and proboscis (where most of them are found upon dissection). The caudal end of *Wuchereria bancrofti* is characterized by having three teat-like papillae of equal size. In the genus Brugia, the central papilla at the caudal end is most prominent, while the two ventro lateral ones are less conspicuous.

The duration of larval development is affected by the ambient temperature; generally, the warmer it is the more rapid the development. It usually takes 10-14 days for W. bancrofti to reach the infective stage, and 7-10 days for B. malayi and B. timori. When the now infective mosquito takes a blood-meal, some or all of the infective larvae escape from the proboscis and actively enter the human host through the wound made by the mosquito. The L3 larva develops in the lymphatic system to the L4 stage, to the young adult stage, and finally to the mature adult worm, male or female. After fertilization, the female worms produce microfilariae, which find their way from the lymphatic system to the bloodstream. The pre-latent period (from the entrance of L3 to the appearance of microfilariae in the peripheral blood) is estimated a: about 3 months for Brugia and 9 months for W. bancrofti.

The Spectrum of Filarial Disease

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

Endemic Normals (EN/UN)

Uninfected, but exposed individuals (asymptomatic amicrofilaremia or endemicnormals)

In endemic areas a considerable proportion of the population remains uninfecteddespite exposure to the parasite [WHO Report]. This group has been termed endemicnormals. The incidence of endemic normals in a population ranges from 0 % to 90 % indifferent endemic areas (WklyEpidemiol Rec).

Asymptomatic Individuals (INF)

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

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

Clinical manifestations in expatriates

The clinical manifestations in individuals who move from non-endemic areas to endemic areas are characterized by the rapid appearance of signs and symptoms which are not commonly seen in endemic populations. They rapidly develop inflammatory pathology. These lesions respond rapidly to specific treatment and more importantly can be reversed when the individuals return to their non-endemic environment.

The microfilaremic stage

This is characterized by the presence of microfilariae in the peripheral blood and until recently these individuals were classified as "asymptomatic individuals" since they had no overt clinical manifestations of filariasis. This was thought to be related with their immunologically down-regulated state. However, two sets of recent observations have revealed this being clinically "asymptomatic' in no way implies freedom from morbidity. First, it was recognized that most of these microfilaremic individuals have haematuria and/or proteinuria that reflects low-grade renal damage which does appear generally to be reversible after treatment. Second, and even more dramatic, were the observations by several groups of investigators using lymphoscintigraphy to visualize by radioisotope tracer techniques the functional anatomy of lymphatic vessels. These individuals, even though asymptomatic, had markedly abnormal, dilated lymphatics and markedly abnormal patterns of lymphatic flow. Though reversibility with treatment has not yet been demonstrated, it is clear that the "asymptomatic microfilaremic state" is not so benign as initially believed. Some individuals remain asymptomatic for years, while others progress more rapidly to the acute and chronic stages.

The acute manifestations

The acute clinical manifestations of filariasis are characterized by recurrent attacks of fever associated with inflammation of the lymphnodes (lymphadenitis) and lymph vessels (lymphangitis) termed as adenolymphangitis (ADL). In bancroftianfilariasis, recurrent attacks of fever associated with lymphadenitis are less frequently seen than in Brugianfilariasis. In addition to the lymph nodes in the inguinal, axillary and epitrochlear regions, the lymphatic system of the male genitalia is frequently affected, leading to funiculitis, epididymitis or orchitis, or to a combination of these.In *Brugian*filariasis, the affected lymph nodes are mostly situated in the inguinal and axillary regions, with inflammation along the course of the distal lymphatic vessels. Quite often, an attack of lymphadenitis is precipitated by hard physical work.

The role of bacterial/fungal infections in ADL attacks

Recent evidence, both from clinical observations and from immunohistological and bacteriological studies of tissue from lymphoedematous limbs of affected patients, has suggested that bacterial or fungal superinfections of limbs with compromised lymphatic function play the primary role in triggering most episodes of ADL, which, themselves, actually cause or exacerbate the elephantiasis changes in affected patients.

The course of acute attacks

Most workers now agree that it is possible to distinguish two forms of acute attacks. The first type the so-called "filarial" fever is characterized by lymphadenitis and retrograde lymphangitis in the absence of any injury or entry site for bacterial infections. The second form is usually diagnosed as cellulitis and is almost always associated with a visible site of entry for bacteria. Oedematous infiltration of the

surrounding subcutaneous tissues or even formation of abscesses, may in turn ulcerate and lead to scarring. In contrast to bacterial infections, the ulcer in filariasis is relatively clean, and produces a serosanguinous fluid. Lymphoedema is quite often present in these fulminant episodes. Usually, the oedema subsides after each episodic attack, but with repeated attacks the oedema persists, leading to chronic lymphoedema. Typically, each attack of fever and lymphadenitis lasts for several days and usually subsides spontaneously following bed-rest.

Adenolymphangitis (ADL)

ADL present as localized [Bonnetblanc J et al]. Contributing to an ongoing problem concerning the development of sound data to understand the effects of drug administration on the effects of LF is a lack of consensus among researchers on how to define ADL or "acute attack." Unlike evidence of chronic infection, ADL proves less specific and more varied in terms of inflammation of the skin with involvement of the nodes and lymphatic vessels accompanied by fever . Typically, ADL lasts from a few days to one to three weeks at a time and may occur multiple times per year as episodes of extreme pain [Bonnetblanc J et al]. Contributing to an ongoing problem concerning the development of sound data to understand the effects of drug administration on the effects of LF is a lack of consensus among researchers on how to define ADL or "acute attack." Unlike evidence of chronic infection, ADL proves less specific and more varied in terms of duration, physical presentation, and symptoms. There is longstanding debate on the extent and pathogenesis of ADL. From clinical data in the 1940s, LF morbidity was originally grouped into primary, secondary or tertiary filariasis: a primary case developed acute filarial fever or lymphangitis; secondary cases occurred at adenopathy onset; and tertiary cases yielded hydrocele and elephantiasis [Gyapong JO et al]. However, this original division proved problematic given that clinical

manifestations often overlap one another and symptoms of acute disease, chills, fever and malaise, easily mimic a plethora of disease states endemic to the geographical regions with ongoing control programs.

Studies into the 1950's divided clinical LF into either acute or chronic cases. Hewitt's study in British Guiana on MDA and clinical disease first reported acute cases as demonstrating lymphangitis, lymphadenitis, filarial fever, orchitis, abscess, or severe abdominal pain at follow up after administration of DEC [Ryan Tet al]. Most of the early studies evaluating MDA and ADL have included lymphangitis and acute filarial fever as representative of ADL while defining the onset of enlarged glands as chronic in nature [Noroes J et al , Gyapong JO et al , Alexander N et al]. Starting with Ciferri in 1969, further research involving ADL began using adenolymphangitis[Rao CK et al , Kanda K et al] as an identifying marker. At no point do any of the previous studies distinguish attacks based on underlying biology or describe the distinguishing features between lymphangitis and adenolymphangitis.

Many etiologies have been postulated as an underlying reason for ADL; causes have included secondary bacterial infections, immune system response to filarial antigens, and reactions mediated by the living and/or deceased adult worm [Dreyer G et al]. These events are thought to contribute to edema progression and the exacerbation of physical impairment [Babu BV et al]. Originally acute filarial lymphangitis (AFL) was thought to originate from the body's immune defense against dying adult worms or the result of an allergy against the filarial larvae parasite; a review by Addiss et al. described the lympangitis progressing distally along the lymphatic vessel producing a palpable cord with accompanying symptoms of fever, headache and malaise [Dreyer G et al]. Now, further research has revealed the role of infection, particularly Group A

streptococcus, as a major contributing factor to the origin of acute events and the eventual development of lymphedema [Shenoy RK et al, Vijayalakshmi N et al, Baird Jet al]. Symptoms due to dermal conditions, similar to cellulitis of the extremities, are grouped under the term acute dermatolymphangioadenitis (ADLA). With this expanded knowledge of the need to prevent secondary infection in order to avert ADL, morbidity management programs have become increasingly aware of the need to emphasize cleansing infected areas and attempting to minimize damage progression with treatment options including topical and oral antibiotics [Anderson J].

Even today, however, resolution has not been reached over the comprehensiveness of acute disease description. Currently there is still question over incorporating a case definition for an associated 3rd stage larva induced lymphangitis, which does not fit into either the AFL or ADLA category [Richard SA et al]. Ideally, future research into clinical morbidity will utilize a standardized definition based on the latest known data explaining the etiologies for each unique presentation underlying acute disease, however this may involve intense clinical monitoring, bacterial culture, treatment, and diagnostic studies to determine worm death [39]. Regardless, correct clinical determination of ADL depends on establishing a consistent methodology of data collection.

Chronic Pathology (CP)

The chronic sequelae of lymphatic filariasis develop years after initial infection [Partono F et al]. InBancroftian filariasis the main clinical features are hydrocele, lymphedema, elephantiasis andchyluria. The manifestations are hydrocele and swelling of the testis and/or lymphedema ofthe entire lower limb, the scrotum, the entire arm, the vulva, and the breast [Pani et al,]; on the otherhand, **i**n Brugian filariasis the leg

below the knee and the arm below the elbow are commonlyinvolved but not the genitals. Therefore, the development of pathology is thought to bedependent on the presence of the adult worm. Histologically, the worm elicits little reaction aslong as it is alive; however, upon death of the adult worm, a granulomatous reaction ensues [Dreyer G et al]. The granulomas are characterized by macrophages (which develop into giant cells),plasma cells, eosinophils, neutrophils and lymphocytes. There is endothelial and connective tissue proliferation with tortuosity of the lymphatics and damaged or incompetent lymphvalves. This typically results in lymphatic dilatation and subsequently lymphatic dysfunctionand compromise leading to lymphedema. Early pitting edema can give rise to subsequentbrawny edema with hardening of tissues and later hyper-pigmentation and hyperkeratosis withwart-like protuberances, which, on histological examination, reveal dilated loops of lymphaticvessels within nodular lesions. Very important in the progression of these lesions is the factthat redundant skin folds, cracks and fissures in the skin provide havens for bacteria and fungi to thrive and intermittently penetrate the epidermis leading to either local or systemic infections. Sometimes the skin over the nodules breaks down causing the dilated lymphaticwithin to rupture and discharge lymph fluid directly into the environment, at the same timeserving as a pathway for entry of microorganisms into the lymphatic [Olszewski WL et al]. In men scrotal hydrocele is the most common chronic clinical manifestation of bancroftian filariasis [Partono F et al. ; Dreyer G et al]. It is uncommon in childhood but is seen more frequently postpuberty and increases in incidence with age. In some endemic communities, 40–60 % of alladult males have hydroceles. Hydroceles are due to accumulation of edematous fluid in thecavity of the tunica vaginalis testis. Though the mechanism of fluid accumulation is unknown,direct ultrasonographic evidence indicates that in bancroftian filariasis the scrotal lymphaticsare the preferred site of

localization of the filarial worms and their presence may stimulate notonly the proliferation of lymphatic endothelium but also a transudation of hydrocele fluidwhose chemical composition is not dissimilar to serum. Chronic epididymitis and funiculitis can also occur. The prevalence of chyluria (excretion of chyle) is very low.

Other manifestations

Lymphatic filariasis has been associated with a variety of renal abnormalities includinghematuria, proteinuria, nephrotic syndrome and glomerulonephritis [Melrose WD et al]. Circulating immunecomplexes containing filarial antigens have been implicated in the renal damage. Lymphaticfilariasis may also present as a mono-arthritis of the knee or ankle joint [Adebajo AO et al].

Pathogenesis of Filarial Disease

The most severe clinical manifestations of lymphatic filariasis are lymphedema and elephantiasis. Although the immune responses to filarial parasites have been well studied with respect to natural history, diagnosis and treatment, there is a relative paucity of information in terms of the mechanisms underlying development of pathology.

The two major independent components of lymphatic filarial disease are lymphangiectasia and inflammatory reactions. While most infected individuals exhibit lymphangiectasia, clinically apparent lymphedema may not be common [Freedman DO et al, Dreyer G et al].It is also clear that with patent infection, lymphangiectasia develops in the vicinity of adult worm nests [Dreyer G et al]. Subclinical lymphangiectasia of the lymphatic vessels containing live adult worms have been shown to exhibit distention with no apparent inflammatory reactions in the vessel wall, with little or only a fleeting inflammatory response to living adult parasites [Dreyer G

et al]. Further, the fact that lymphangiectasia is not restricted to the exact segment of lymphatics where the worms reside [Amaral F et al, Dreyer G, et al] suggests that this process is mediated by soluble products excreted or secreted by the parasite that act on the lymphatic endothelial cells. It is also clear that with the advent of adaptive immunity, the host inflammatory response against the dead or dying worm and the subsequent release of parasite products and inflammatory mediators, a stage of irreversible lymphatic dysfunction ensues [Figueredo-Silva J et al, Connor DH et al , von Lichtenberg F et al]. This then manifests clinically as progressive lymphedema. In addition, lymphatic dysfunction has been shown to predispose infected individuals to secondary bacterial and fungal infections and trigger inflammatory reactions in the skin and subcutaneous tissue that accelerates the progression of lymphedema and precipitates the development of elephantiasis [Olszewski WL et al, Shenoy RK et al].

Cells of the innate and adaptive immune system are important for the initiation of type 2 immunity, which are the hallmark of helminth infections. The key players in T helper (Th) 2-type immunity are CD4+ Th2 cells and involve the cytokines—IL-4, IL-5, IL-9, IL-10, and IL-13; the antibody isotypes—IgG1, IgG4, and IgE, and expanded populations of eosinophils, basophils, mast cells, and alternatively activated macrophages [Allen JE and Maizels RM ,McSorley HJ, Maizels RM].

The importance of pro-inflammatory cytokines, possibly of innate origin, in the pathogenesis of lymphedema, has been strengthened by a series of studies in humans with chronic pathology, either in early or late stages or lymphedema. Studies have shown that individuals with chronic lymphatic pathology have elevated levels of C-reactive protein (an acute phase protein, indicating an acute inflammatory response) [Lal RB et al] pro-inflammatory cytokines such as TNF-α, IL-6 and soluble TNF

receptor (Das BK et al, Satapathy AK et al), endothelin-1 and IL-2 (el-Sharkawy IM et al 2001), as well as IL-8, MIP-1α, MIP-1β, MCP-1, TARC and IP-10 in the peripheral circulation. Similarly, while patients with both acute and chronic manifestations of LF have elevated circulating levels of IL-6 and IL-8, only those with chronic disease manifestations have elevated levels of sTNF receptors [Babu S et al].

The endothelium appears to be closely associated with pathogenesis of lymphatic disease, studies targeting the interaction between endothelial cells (vascular or lymphatic) and filarial parasites have been performed. Differentiation of LEC into tube-like networks was found to be associated with significantly increased levels of matrix metalloproteinases (MMPs) and inhibition of their endogenous inhibitors—TIMPs (tissue inhibitors of MPs). [Bennuru S et al].Recent data suggest that an increase in circulating levels of MMPs and TIMPs is characteristic of the filarial disease process and that altered ratios of MMP/TIMP are an important underlying factor in the pathogenesis of tissue fibrosis in filarial lymphatic disease. [Anuradha R et al] Other studies have implicated the vascular endothelial growth factor (VEGF) family in lymphangiogenesis. [Pfarr KM et al, Debrah et al] Other angiogenic factors such as angiopoietins-1 and -2 are also found at elevated levels in individuals with filarial-induced pathology. [Bennuru S et al], A major factor involved in the initiation of the proinflammatory response and the increased production of VEGF-A and -C might be the endosymbiont, Wolbachia, present in most filarial nematodes (including W. bancrofti and the 2 Brugia spp) [Pfarr KM et al]. Recently, it has been demonstrated that the increased levels of VEGF-C and sVEGF-R3 (observed in lymphedema patients) were reduced following doxycycline treatment (a regimen that eliminates Wolbachia) and that there was improvement in lymphedema. [Debrah et al].

Persistent immune activation is associated with elevations of circulating microbial products, acute-phase proteins, and the so-called microbial translocation molecules. [Brenchley JM et al] however, intra and peri-lymphatic damage—an underlying feature of filarial disease—might also contribute to the presence of microbial translocation products in the bloodstream. Indeed, the increased circulating levels of LPS (which serves as a marker for microbial translocation) and decreased levels of LPS-binding protein (LBP) are characteristic features of filarial lymphatic pathology [Anuradha R et al] that in turn appears to cause immune activation. Since filarial lymphedema is known to be associated with increased bacterial and fungal loads in the lymphatics, our studies reveal that these damaged lymphatics may serve as a potential nidus for bacterial translocation through leaky lymphatic endothelium.

Multi-color flow cytometry analysis reveals that the frequency of Th1 cells (CD4+ T cells expressing either IFNγ or IL-2 or TNF-α) [Anuradha et al]; Th9 cells (CD4+ T cells expressing IL-9 and IL-10) [Anuradha et al]; Th17 cells (CD4+ T cells expressing IL-17) [Anuradha et al] and Th2 cells (CD4+ T cells expressing IL-22) is significantly enhanced in filarial pathology. This is accompanied by a concomitant decrease in the frequency of Th2 cells (CD4+ T cells expressing IL-4 or IL-5 or IL-13) both at homeostasis and following parasite antigen stimulation [Anuradha et al]. Although less well studied than Th1 cells, Th17 cells might also have an important role in the pathogenesis of disease in filarial infection since PBMC from individuals with pathology (but not asymptomatic patients) express significantly higher levels of the Th17 associated cytokines as well as the master transcription factor - RORC at the mRNA level [Babu S et al].

Finally, pathology in lymphatic filariasis is also associated with expanded frequencies of Th9 cells, CD4+ T cells that express both IL-9 and IL-10 but not IL-4 and this frequency exhibits a positive correlation with the severity of lymphedema in filarial infections [Anuradha et al].

DIAGNOSIS AND TREATMENT

Acute Filarial disease

In patients with filarial disease acute attacks of adenolymphangitis (ADL) may involve the limb, breast or male external genitalia

These acute episodes are characterized by local

1) Pain
2) Tenderness
3) Warmth
4) Lymphadenitis and or lymphangitis

Other commonly associated findings include fever, oedema, constitutional complaints and localized or ulcerated abscesses especially in areas where Brugia is endemic. In endemic areas there are two distinct types of acute ADL episodes

a) ADL secondary to bacterial or fungal infection
b) ADL caused directly by the parasite itself

Acute attack secondary bacterial or fungal infection:

This is the commonest form of ADL. It is usually recognized as a syndrome with a toxic picture that can include high fever, chills, myalgia and headache. Oedematous inflammatory plaques clearly demarcated from normal skin are usually

seen. Occasionally vesicles, ulcers and hyper-pigmentation may also be noted. There is often a history of trauma, burns, radiation, insect bites, punctiform lesions or chemical injury. Entry lesions especially in the interdigital area are common. This condition responds to treatment with antibiotics and hygiene.

Filarial acute attacks

The commonest presentation is that of a cord-like structure associated with retrograde lymphangitis in the lower or upper limbs. In the scrotal area or the breast it may present as a painful palpable nodule. Funiculo-epididymoorchitis is the usual presenting feature of acute attacks of ADL involving the male genitalia. The patient is in pain and on examination the scrotum is painful and red with testicular •enderness. Acute hydrocoele may also be present. The systemic reactions arc mild and distal edema is rare. Recurrence of these attacks at the same site is common.

CHRONIC DISEASE

1.Lymphoedema of the limbs

Swelling of the limbs is most common. In bancroftianfilariasis the entire limb may be affected, but in Brugianfilariasis, the leg below the knee and sometimes the arm below the elbow are characteristically involved

Lymphoedema can be graded as follows:

Grade 1 : Pitting edema of the limb that is reversible on elevating the limb.

Grade 2 : Pitting/Non pitting oedema that is not reversible on elevating the limb. Skin is normal

Grade 3 : Non-pitting oedema of the limb. Not reversible on elevation. Skin is thickened.

Grade 4 : Non-pitting oedema with fibrotic and verrucous skin changes(elephantiasis) (Fig.4)

Scars

The presence of scar tissue, around the groin or elbow resulting from previously ruptured abscesses, is a helpful sign supporting the clinical history of ADL episodes; it should therefore be looked for carefully.

2. Male Genital Disease: (seen only in Bancroftianfilariasis) Hydrocoele

An increase in the size of the scrotum with a collection of clear fluid. Small hydrocoeles are difficult to diagnose. Transillumination with a pen-torch in a dark room is characteristic and quite often very helpful to identify fluid. It should be noted that the two testicles are seldom equal in size; one may be considerably larger than the other, simulating a small hydrocoele. Hydrocoeles should be differentiated from inguino-scrotal swellings and hernias. For field purposes, the following grading is recommended for hydrocoeles:

Grade I	-	smaller than a tennis ball
Grade II	-	in between grades I and III
Grade III	-	bigger than the patient's head.

Lymphoedema of the genitalia

Swelling of the scrotum and/or with thickened scrotal or penile skin which may have characteristic "peaud'orange" appearance. In long standing cases verrucous lesions and lymphorrea are common.

Lymphorrhea

In this condition lymph oozes out to the exterior direcdy from dilated ruptured lymphatic vessels. The dermis may be normal. This frequently occurs in the scrotal wall.

Chyluria: (the presence of chyle in the urine, giving it a milky appearance)

Patient complains of passing milky urine. Chyluria is more frequently observed after a hyperUpidemic meal and may in many cases be associated with blood in the urine (haematochyluria).

To make a diagnosis of chyluria, collect urine in a clear container.

Examine for the presence of lymphocytes under the microscope. Chyluria should be differentiated from pyuria (pus in the urine) and phosphaturia. In these conditions, white blood cells are absent and a sediment rather than an emulsion isseen.

Tropical Pulmonary Eosinophilia

The usual presenting features are cough, dyspnea, wheezing similar to bronchial asthma. Chest X-ray may show diffuse mottling, but this is not pathognomonic. The total eosinophil count is greater than 2000/mm^3. Increasedlevels of IgH and anti-filarial antibodies are commonly found. Similar syndromes may occur in the presence of intestinal worm infections.

Assessment of magnitude of lymphatic filariasis

Data on prevalence of elephantiasis or hydrocoele can be obtained from the following sources: In urban areas; from hospitals, private physicians and other health services; - in rural areas: from health services, private physicians or village chiefs.

RAPID ASSESSMENT PROCEDURES

Sociological approaches

Key person interviews, focus group discussions and self-administered questionnaires: These techniques have been successfully used to identify high risk communities and providing relevant information for planning a control programme. They have the advantage that they are convenient and quick to use, non-invasive and relatively inexpensive (cost-effective) and valid (at least in endemic areas in Africa). Key informants usually include individuals who have access to information in the village or community (village chief, school teachers, postman, barber). Semi-structured interviews are used to gather information regarding the terms used to define filarial disease, knowledge of the cause and transmission of filariasis, health seeking practice regarding the disease and perceptions of the burden of the disease in terms of distribution, numbers and severity and focus. This information is used to for drawing up guidelines for focus group discussions. Commonly used groups include women's groups, men's groups and people with chronic disease. All interviews are transcribed and analysed using software programmes (Text Base Alpha) that can analysequalitative data.

Male genital pathology and microfilaremia

The prevalence of hydrocoeles in a community estimated by random examination of 30-40 adult males above the age of 20 years per community was found to correlate very well with the community prevalence of microfilaremia. This may be used to rapidly assess the prevalence of filariasis in a community and also determine the microfilaria rates. It is possible to detect living adult worms and small hydrocoeles using the commonly available 3.5 mHz probe

PARASITES

Identification in the blood

a) Blood films

The examination of blood films is the method most widely used for field surveys. If properly done, it is a reliable method for qualitative and quantitative microfilarial studies. It is recommended that a measured amount of blood (at least 20µl and preferably 60 µl, to be smeared in 3 rows of 20 µl each on the same side) be used. Technicians employed in filariasis surveys should be regularly tested as to their skill in comparison with senior member of the staff who has great experience, or with each other. The same blood films with microfilariae are examined by each person separately.

b) Nuclepore filtration technique

This technique can be used for counting microfilariae as well as making a diagnosis. A 3µm filter is preferred. The sensitivity of the test is dependent on the volume of blood used.

c) Knott's concentration technique

1 ml of venous blood is mixed with 10 ml of dilute formalin (20 ml/litre) in a conical centrifuge tube. Leave the tube in an upright position for 12 hours or centrifuge for 5minutes at 350g. Pour of the supernatant. Examine the sediment for microfilariae. A few drops of 1% methylene blue may help in showing up the microfilariae.

A disadvantage of this technique is that it is laborious and the precipitate has jelly-like consistency which sometimes makes further examination difficult.

Antigen Detection Assays

None of the immunodiagnostic tests that are dependent on antibody detection currently available are able to define accurately the presence of infection, either because of lack of specificity or because of inability to discriminate between present and past infection. However, the absence of antifilarial antibodies in a patient residing in a nonfilarial endemic area excludes the possibility of a filarial etiology. More recent diagnostic tests such as the ICT test have detected protein antigens whose peptides react with the monoclonal antibodies which are bound on a card. These assays which are easy to perform detect circulating antigens in sera from essentially all microfilaremic and a proportion of amicrofilaremic persons residing in W. bancrofti endemic areas. Importantly, the levels of circulating antigen appear constant throughout the day (unlike microfilaremia) and fall to zero after successful chemotherapy has killed the adult worms. No comparable assays exist for B. malayi infections.

These assays detect circulating antigens in sera from essentially all microfilaremic and a proportion of amicrofilaremic persons residing in W. bancrofti endemic areas. Importantly, the levels of circulating antigen appear constant -hroughout the day (unlike microfilaremia) and fall to zero after successful chemotherapy has killed the adult worms. No comparable assays exist for B. malayi infections.

Filariasis Test Strip

A new diagnostic test enabling countries to determine when to stop large-scale treatment of populations to eliminate lymphatic filariasis is now available. WHO is coordinating the procurement and supply of the new test for use in its Global Programme to Eliminate Lymphatic Filariasis (GPELF).

The Filariasis Test Strip (FTS) is a new point-of-care rapid diagnostic test designed to detect in human blood the antigen of the major species of filarial worm (*Wuchereria bancrofti*) that causes lymphatic filariasis.

Detection of adult worms

Ultrasound

Adult W. bancrofti worms can be detected using ultrasound employing special probes. Probes greater than 5 mHz can easily identify adult worms in the scrotal lymphatics of most microfilaremics and also some individuals who are amicrofilaremic. Adult worms are detected by their characteristic movements (filarial dance sign). The worms are remarkably constant in their location and this technique can be used to monitor success of therapy.

VECTORS

In order to determine which species are the local vectors of lymphatic filariasis or, subsequently, to measure the amount of transmission that is occurring, it is necessary to dissect mosquitoes and examine them for filarial larvae.

Individual dissection

First-stage larvae are small motionless, and have different size and shapes, so that they are very easily missed when examining with a dissecting microscope, especially when the tissue is not covered with a coverslip.

Mass dissection

The mass dissection method is useful since it enables large numbers of mosquitoes of the same species to be examined rapidly and easily to determine the

numbers of infective larvae they were harbouring, and thus to estimate the amount of transmission that is occurring.

DNA based diagnostics

DNA based technology can now also be used for diagnosis of filarial infection both in humans and in the mosquito vectors by polymerase chain reaction (PCR)-based assays which provide outstanding sensitivity and specificity. For B. malayi, PCR techniques can detect a single L3 in pools of up to 100 mosquitoes, a single microfilaria in 1ml of blood, or the equivalent of 1 pg of DNA in 100 pi of blood. Recently, a similar PCR assay for W. bancrofti has been developed with similar sensitivity. It is estimated that one technician can now use these assays to screen up to 3600 mosquitoes (36 runs of 100 mosquitoes each) or 1000 blood samples in one day. The current cost for materials (primarily for the enzymes required for PCR) is US $1.00 per run.

The advantages of PCR-based tests include high degrees of sensitivity and species-specificity, their detection of only current infections, and the rapidity with which their results can be obtained (same day). In addition, samples can be preserved at ambient temperature for months and shipped to a central laboratory for assay. The primary drawbacks of this technology are the special training and equipment required, and the need for its performance in a central laboratory with good quality control. In addition, its use in assessing transmission of infection in vectors requires further semiquantitative PCR output to the transmission indices in standard use that ire based on detection of infective larvae in dissected mosquitoes.

CHEMOTHERAPY OF LYMPHATIC FILARIASIS

Parasite Control

W. bancrofti

Diethylcarbamazine citrate (DEC) DEC is generally very safe, and though not encouraged, its use in pregnancy has not been reported to have complications; care should also be taken when treating people with chronic kidney or cardiac disorders.

The recommended dose of DEC for individual treatment is 6 mg/kg of body weight daily for 12 consecutive days (total dose, 72 mg/kg). Repeated course of treatment may be necessary. The drug is rapidly excreted from the body unmetabolised, mainly though the kidneys and the possibility of a cumulative effect is minimal. The excretion of DEC is highly pH dependent. Under acid conditions pH 5) some 70% of the given dose of DEC is excreted unchanged in the urine, while under alkaline conditions (pH 7.5 and above) only 5% of DEC is eliminated as DEC-N-oxide.

B.malayi and *B. timori*

Treatment of with DEC is similar to that for *W. bancrofti* but with the following differences.

(a) Treatment is associated with more severe systemic adverse reactions because of the sensitivity of the parasite to the drug.
(b) The total dose used is generally less. The daily dose ranges from 3 to 6 mg/kg of body weight for 6 to 12 days (total dose from 18 to 72 mg)

Adverse reactions

Three types of adverse reactions may be experienced by the patient when he receives DEC.

1. Pharmacologic side reactions: These are drug-dose dependent and include gastric upsets and drowsiness.
2. Systemic adverse reactions due to the killing of microfilariae: These, in more or less decreasing order of frequency, are fever, headache, body ache, dizziness, decreased appetite, malaise, urticaria, and sometimes bronchial asthma. These systemic side-reactions after DEC are less likely to occur and are less severe in bancroftianfilariasis than in Brugianfilariasis. These are positively associated with microfilariaemia and with the density of microfilariae. They occur early during the treatment and generally do not last more than 3 days.
3. Local adverse reactions (due to adult worm killing) are characterized by adenitis and lymphangitis, funiculitis, epididymitis, orchitis, abscess formation, ulceration and transient lymphoedema. These tend to occur at later stages of post-treatment and may last longer.

Both general and local reactions will disappear spontaneously and usually it is not necessary to interrupt treatment. Symptomatic treatment of systemic adverse reactions with antipyretics and analgesics may be helpful.

Ivermectin

Ivermectin, a semi-synthetic macrolide in single oral doses of 400ug/kg has been shown to be effective in clearing microfilaremia of W. bancrofti and B.malayi. The reactions that occur post-treatment are essentially similar to those seen with DEC.

Albendazole

More recently it has been demonstrated that combination of albendazole with DEC or Ivermectin can effectively clear microfilaraemia of bancroftian infections (WHO, 1998). Single dose two drug combinations of albendazole plus either DEC or

Ivermectin are superior in efficacy to single drug treatment for decreasing microfilaraemia in lymphatic filariasis. Albendazole alone has shown to possess killing or sterilizing activity on adult filarial parasite (Denham et al., 1980; Mak et a/., 1984). Albendazole plus DEC/Ivermectin co-administration offers "beyond filariasis" benefits in terms of comprehensive health development and cognitive function in children. Also, addition of albendazole to DEC did not appear to increase the frequency or intensity of adverse events when compared to DEC when given alone (WHO, 1999). However, the usefulness of this approach needs to be evaluated on large scale before itsupscaling to cover the entire population at the risk of filarial infection.

Control programmes

The focus of control efforts should be on treating the infection in human populations, with vector control serving a supporting role when feasible and affordable. "Mass-distribution" programmes should completely replace those based on a 'selective treatment' strategy. Co-administration of two drugs is recommended.

Mass treatment

The concept behind this strategy is that, in an area of high endemicity, everyone is more or less equally exposed to the infective bites of the vector, and available methods are not sufficiently sensitive to diagnose sub-latent or subclinical infections. Therefore, in mass treatment, drugs arc given to almost everyone in the community irrespective of whether or not they have microfilaraemia or disease manifestations. However, the drug should not be given to infants, pregnant women, the elderly, or those with obvious debilitating disorders, especially those with cardiac or kidney disease. The advantages of mass treatment are as follows:

a) It is not necessary to examine every member of the community. If the target population is large, examination of a statistically appropriate sample will give a valid estimate of the filarial endemicity of the community.
b) It is not necessary to use a highly sensitive method to detect microfilaria-positive individuals in the community.
c) There is no need to worry about false negative results, which may be due to the insensitivity of the method, inappropriate blood collection or processing, or technical errors.
d) In mass treatment, the chance of not giving the drug to a person occurs only once, i.e., during the distribution of the drug. In selective treatment, the chance of missing a person occurs twice: once during selection of diseased and microfilaria-positive individuals and again during the distribution of the drug.
e) Mass treatment avoid the problem of discrimination whereby some microfilarial carriers, feeling themselves to be in perfect health, may wonder why they have been selected for treatment, while amicrofilariaemic people may wonder why they are excluded from receiving the medicine.

It is now clear that "Mass-distribution" programmes should completely replace those based on a ‘selective-treatment’ strategy (i.e., detection of microfilaremics who are then treated ‘selectively’).

Recent findings about optimal ways in which DEC and Ivermectin and albendazole can be used (alone and in combination) have helped to formulate newer strategies for parasite control in both Brugicm and bancroftianfilariasis. DEC and Ivermectin are primarily microfilaricidal drugs, though it is clear that DEC there is macrofilaricidal activity as well. Moreover, even if these drugs had only

microfilaricidal effects, success should still be anticipated in control programmes where they are used, because prolonged clearance or decrease of microfilariae from the blood helps to reduce transmission of infection, and because reduced transmission and decreased levels of microfilaremia in the community have long been recognized to have a positive "clinical effect" on subjects (i.e., decreased frequency of ADL attacks which lead to decreased incidence of clinical manifestations, and thus enhanced compliance in community programmes). The use of DEC in salt is a special form of mass treatment using very low doses of the drug over a long period. Common salt medicated with l-4g of DEC per g of salt has been used for control in several areas in which W. bancrofti or B. malayi is endemic. Administration of DEC-medicated salt is simple, rapid, safe, inexpensive, efficient,prophylactic, and practical for filariasis control or, in some circumstances, eradication.

A variety of regimens are available for parasite control.

1) Standard 12-day (W. bancrofti) or 6-day (B. malayi) courses of DEC

This is usually administered repeatedly - often at one year intervals-as mass treatment to affected communities. In fact, these regimens have been widely used in many endemic countries. Such regimens, however, have proven to be expensive and difficult to administer both because of the drug's causing rapid parasite death that leads to fever and malaise (systemic adverse reactions), local inflammatory reactions (localized adverse reaction) or gastrointestinal symptoms (the major DEC pharmacological "side effect") in many who were initially microfilaremic but entirely asymptomatic. Indeed it is largely because this therapeutic regimen was so unpopular that alternative treatment regimens have been sought. Furthermore, while the same "standard courses" of DEC have also been used in treatment programmes focused on

"selective chemotherapy" where only microfilaremic individuals were treated with this strategy, too, has proven to be cumbersome and unworkable both for the reasons affecting mass treatment programmes and because of the additional resources necessary to carry out diagnostic procedures on the entire population in order to identify the microfilaremic individuals requiring treatment. Thus "standard-course DEC" can be effective for mass chemotherapy but at a cost in resources, health personnel and patient compliance that makes it impractical for most control programmes.

2. Single-dose ("spaced dose") DEC given at weekly, monthly, 6-monthly or yearly intervals has been enthusiastically advanced for many years in some endemic countries. Recent controlled clinical trials have confirmed the efficacy of such regimens. While more frequent single-dose (usually weekly or monthly) regimens can be effective in decreasing micro filarial prevalence and density, their advantage over yearly or 6-monthly DEC may not be great enough to warrant the increased expense of more frequent drug delivery. For bancroftianfilariasis, it is impressive that these regimens even though lacking complete coverage of the population at each round of treatment, yielded reductions in microfilarial densities that approximate those seen when individuals have been treated with single doses (or with the 12 day standard course) of DEC and followed sequentially for 12 or more months (reductions in microfilarial density of 92-96% at 1 year).

3. Single-dose Ivermectin has been used in large-scale community control programmes for lymphatic filariasis, but its effectiveness against microfilariae of both W. bancrofti and B. tnalayi has been evaluated in individual patients for periods of 12-24 months after drug administration. Numerous earlier studies had examined the effectiveness of lower Ivermectin dosages, but it is clear now that dose of 400 ug/kg

yields definitely superior results. While microfilarial prevalence fell by only 36-70% at 12 months post-treatment, this dose decreased microfilarial densities by 86-99% for 12-24 months post-treatment in both W. bancrofti and B. malayi infections. Thus, since single yearly (or even 2-yearly) doses of Ivermectin appear equally as effective as similar dosing with DEC, Ivermectin alone would be a valuable alternative control tool for use in endemic communities, especially where the use of DEC is contraindicated (as in areas where onhocerciasis or loaisis coexists).

4. The combination of single doses of DEC and Ivermectin with albendazole:

This combination appears to be significantly more effective than either drug alone. At 12 and 24 months post-treatment W. bancrofti infected patients receiving combination showed a fall in microfilarial prevalence of 45-70% and a decrease in microfilarial density of 96-99%. Furthermore, Ivermectin used in doses as low as 20 g/kg (with 6 mg/kg of DEC) was capable of achieving the same effect as the 400 g/kg dose used singly. Thus the potential value of the combination of Ivermectin and DEC with albendazole for use as a chemotherapeutic tool appears most promising.

5. DEC-fortified salt : DEC in concentrations ranging from 0.1-0.6% can be used as a substitute for normal cooking and table salt since DEC is chemically stable. When consumed for periods of 6-9 months it has regularly decreased microfilarial prevalence by 70-100% in both bancroftian and Brugianfilariasis. DEC -fortified salt has been used as the mainstay for control programmes in very large populations in China, Taiwan and India, with excellent results that substantiate observations made on patients followed individually and in whom prevalence of W. bancrofti has been shown to decrease by 97% after 4 months of DEC-salt usage and whose microfilarialdensities fell even more dramatically, by greater than 99%. Though this strategy of DEC-salt usage does appear

both workable and highly effective, essentially all of the communities in which it has been employed thus far have only had access to salt supplies that were strictly controlled by health care authorities.

Regimens recommended for mass treatment in areas where there is no co-existing onchocerciasis or loaisis would be either of the following:

a) Ivermectin [150 (g/kg) or DEC (6mg/kg)] in combination with Albendazole (400 mg) given once yearly for 5-7 years.
b) Single, annual or semi-annual mass administration of DEC (6mg/kg body weight) for 5-10 years.
c) DEC fortified salt (0.2-0.4%) used in place of regular salt for all cooking and seasoning for a period of 9-12 months.

Regimen recommended for mass treatment in areas where there is co-existing onchocerciasis or loaisis. Ivermectin (150mg/kg) plus Albendazole (400 mg) given once yearly for 5-10 years. In areas where there is no onchocerciasis the preferred combination is DEC (6mg/kg) in combination with albendazole (400 mg).

Treatment of Brugiamalayi and B. timori with DEC is similar to that for W. bancrofti but with the following differences.

(a) Treatment is associated with more severe side-reactions.

There is consistency in the daily and total doses of DEC commonly used in different countries where B. malayi is endemic. The daily dose ranges from 3 to 6 mg/kg of body weight and the total dose from 18 to 72 mg/kg of body weight. Ivermectin does not clear the microfilariae of Brugianfilariasis as effectively it kills the microfilariae of W. bancrofti.

Morbidity control

The potential for morbidity control has been greatly advanced in recent years by increased understanding of both lymphoedema and acute adenolymphangiii> (ADL) in patients living in filariasis endemic areas. Bacterial or fungal superinfection of limbs with compromised lymphatic function play the primary role in triggering most episodes of ADL which themselves actually cause or exacerbate the elephantiasis changes in affected patients.

A major implication of this new understanding is that simple measures of hygiene, coupled with local (or in severe cases, systemic) antibiotics given prophylactically, can have profound effects in preventing these damaging episodes of ADL and even in allowing the host repair and recover from some or all of the oven damage caused by filarial infection and subsequent superinfections.

Prevention of Acute disease

Acute episodes of adenolymphangitis which are caused by bacterial infections can be prevented by simple hygiene. This usually includes:

1. Avoiding barefoot walking
2. Cleaning the affected limb twice daily with soap and water
3. Keeping the affected limb dry
4. Clipping the nails
5. Applying salicylic acid ointment of webs of the toes, nails and sides of the feet every night
6. Prophylaxis with systemic antibiotics may be necessary for long periods. Acute attacks due to the death of the adult worm cannot be prevented.

Treatment

The same measures of hygiene listed above need to be followed.

It is generally advised to rest the limbs and if possible keep than elevated. Apply cold bandages made using cold water or potassium permanganate solutions (1/10,000 on the scrotal wall and 1/20,000 for other sites). All entry lesions must be treated with broad spectrum topical antibiotics. In severe cases, systemic antibiotics may be necessary. Penicillin is the drug of choice if the patient is not allergic to the drug.

Management of Chronic disease

Progression of chronic disease is related to the repeated occurrence of acute episodes of adenolymphangitis (ADL). A simple treatment programme (Dreyer et al. 1994) can prevent the damage from getting worse and can actually reduce the swelling. The success of the treatment depends on the compliance of the patient.

The basic components of the programme include:

1. Keeping the leg elevated at night for 1 to 2 hours each day
    a. To decrease the swelling at night, patients should be advised to sleep with the foot of the bed raised as high as is comfortable.
    b. During the day they must be encouraged to sit with the leg elevated for at least one hour.
2. Simple exercises for the feet and ankles which involve dorsiflexion, plantar flexion at the ankle and flexion and extension at the knee can be taught to the patient. They should perform them at least one or two times a day.
3. Keeping the legs clean with washing soap and water.

a. Patients should be taught to wash their legs with soap and water every day to keep the skin healthy and prevent infection. It is important to use water that is clean and soap that is gentle on the skin.
b. Mineral oil or skin care lotion must be applied every day to keep the skin of the legs and feet from getting too dry.

4. Preventing infection of feet and legs
    a. The patient must observe the leg and feet every day for signs of inflammation, swelling, or infection and for cracks or lesions of the skin particularly between the toes.
    b. Patients must be instructed that after washing the feet, they must ensure that the space between the toes is completely dry.
    c. They must be taught to apply anti-fungal cream twice a day. If cracks develop in the skin between the toes.
    d. They must avoid walking barefoot.
    e. They should not use any medicines or creams on their legs andfeet without first checking with their doctor. They should notuse methylate or mercury compounds.
    f. Patients must take good care of their toenails. They must bekept trimmed but not too short. Care must be taken to avoid cutting the skin.
    g. Prophylactic antibiotic therapy is recommended for those patientswho have recurrent infections.
5. Prompt treatment of any infections that develop.

    Patients must be instructed contact their doctor as soon as their leg becomes swollen or painful or if they develop an "acute attack".

    If the doctor is not immediately available, the patient should stay in bed with the

leg elevated (except when necessary, for example, going to the bathroom). The affected leg may be cleaned with soap and water and cold compresses using potassium permanganate may be employed.

6. Massage

   This can help move the fluid out of the leg. This may require training by a physiotherapist.

7. Wrapping the leg

   Proper wrapping will help the affected leg from swelling. Wrapping the leg is particularly important when patients are on their feet for a long time.

Surgery for chronic disease

The treatment of choice for hydrocoele is surgery. Several procedures are known to be successful and the most common ones include eversion or excision of the sac.

In some endemic countries plastic and vascular surgeons have carried out shunt procedures (nodo-venous or lympho-venous shunts) to divert the lymphatic flow through venous channels. This is usually followed up with a debulking procedure in an effort to reshape the deformed limb.

Programme on Elimination of Lymphatic Filariasis

Since the inception of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 2000, most of the attention on disease elimination has focused on the interruption of disease transmission with efforts targeting morbidity taking second stage [WHO Report Weekly epidemiological record 2015]]. However, with mass drug administration (MDA) programs successfully reducing microfilaremia rates in targeted communities, more attention has been placed on the need to follow the long term

disabling effects of filarial disease, namely adenolymphangitis (ADL), hydrocele and lymphedema. This awareness has led to an increased number of studies incorporating morbidity assessment as an outcome for monitoring the success of MDA [WHO Report Weekly epidemiological record 2015]. Additionally, GPELF has increased its efforts regarding response to morbidity related issues. Activities based on morbidity prevention have involved educational events at local hospitals, community wide trainings, and patient care tutorials within the context of complex and diverse settings. Most of these actions have occurred as the result of previous research focusing on the benefits of interventions like basic hygiene on preventing physical disease progression. Despite this increase in awareness, morbidity improvement, in particular, has not been considered in depth as a consequence of MDA, and existing literature indicates there is little consistency on how to regard previous research and the best way to approach future assessments in a standardized manner.

From 2000 to 2015 [WHO Report Weekly epidemiological record 2015], 695 million individuals received MDA treatment. Yet, despite such widespread treatment, 40 million people remain plagued with the long-term consequences of LF morbidity. Studies following MDA have generally concentrated on the effects of chemotherapy on microfilaremia levels. Less effort has been devoted to morbidity prevention than hematological elimination, and victims of LF morbidity are often neglected in research concentrating on MDA outcomes. In 2007, a study by Addiss, et al., pointed to the need to prioritize research on the influence of mass antifilarial drug treatment administration on the course of filariasis-associated disease in order to better direct and/or enhance morbidity management strategies. Of the studies on MDA published over last decade, only a few have concentrated exclusively on the specific manifestations of ADL, lymphedema, and hydrocele [Dreyer G et al 2002].

LF related morbidity leads to devastating effects on the health and wellbeing of those afflicted and exacts a heavy toll on the community at large in terms of lost economic productivity. Unfortunately, all residents of endemic areas are susceptible to infection via repeated exposure from mosquito-introduced microfilariae since childhood. With an underlying infection of worms infiltrating the lymphatic system of its victims, the tissue damage exacted by the total worm burden and eventual adult worm death lead to the display of LF signs and symptoms. Restriction of the normal lymph flow causes swelling, scarring, fibrosis and increased susceptibility to infection.

The legs and groin are most affected by the progression of lymphatic damage. As a result, victims are often left disabled and without the resources to understand or deal with the advanced stages of disease. Without providing these individuals with the proper modalities of care, acute inflammatory attacks continue with impunity and thus contribute to lymphedema aggravation and the eventual formation of elephantiasis in 5% of those infected. Similarly, testicular hydrocele characterizes a disfiguring enlargement of the scrotum which can grow to devastating proportions, reducing mobility, limiting work capacity, and inhibiting sexual performance [Anderson J, et al 1996]. Much of the pathogenesis related to LF morbidity is poorly understood, and as a result, misunderstandings of etiology have prevented the rapid evolution of a standardized method to management. However, recent research has accelerated the understanding of how to address morbidity. While more is known regarding biology, there still remains a deficit in knowledge regarding the relationship between MDA and those already afflicted with morbidity.

Previous evaluations dating back to the 1950s regarding MDA have mostly concentrated on the drug diethylcarbamazine, DEC, and its influence on

microfilaranemia as a primary endpoint. This makes sense given the then current tools available to monitor MF blood levels and the fact that DEC has been viewed as the only viable tool in the arsenal of MDA options up until the introduction of ivermectin and albendazole. Thus, the point of earlier investigations has been to evaluate DEC's effectiveness in the fight to interrupt LF transmission in endemic areas at the population level. In addition to evaluating MF levels, studies have also delved into comparing dosing regimens, assessing side effects and tolerability, and, as an aside, effects on clinical morbidity. Since clinical morbidity has rarely served as the topic of main interest, a majority of these studies neglected to formalize a clear and uniform case definition. There is wide heterogeneity in definitions regarding the morbidity of interest, and, interestingly, a lack of consistency on what aspect of clinical morbidity to prioritize. For example, some studies have followed acute disease alone, while others have focused primarily on chronic manifestations, and very few on both. A reason for this results from the follow up periods available to the investigator. With limited funding and time, it is not surprising that the monitoring of chronic disease would take the back burner to more tangible and immediate endpoints. Attempts to follow chronic disease in the form of lymphedema and hydrocele over the short term might not be expected to capture a noticeable difference. Also, the logistics of finding those suffering from transient ADL or relying on a history of symptoms to define ADL does not lend itself to reliable reporting.

The systematic literature review conducted for this thesis explores research regarding GPELF's mission to reduce the burden of morbidity via mass drug administration and what is currently understood regarding the underlying mechanism behind LF induced ADL, lymphedema, and hydrocele morbidities. While the biology of MDA's impact on morbidity has not been extensively researched, there are available

studies with data exploring the effects of MDA on predetermined measures of morbidity (reduction in size, reduction in ADL frequency, or reduction of the incidence of new cases).

Given the fact that GPELF would benefit from sound data in order to be prepared to deal with existing morbidity and given the fact that no study has exclusively concentrated on MDA and filarial morbidity to date, a review of studies relating MDA and morbidity is necessary to assess if MDA, in fact, positively affects morbidity and which medications work the best on a large scale to reduce overall burden. In concert with the goals of the GPELF to address MDA and morbidity as primary targets of interest, research will focus on the comparison and evaluation of studies following the clinical manifestations of lymphatic filariasis after MDA, specifically selecting studies performing a clinical assessment of ADL, lymphedema, and/or hydrocele. Ultimately, this research will provide better insight on how MDA drugs impact LF morbidity.

PREVENTION OF DISABILITIES IN PATIENTS WITH LYMPHOEDEMA

Currently there is no accurate information on the numbers of patients with lymphoedema, hydrocoele and other forms of filarial disease. In many countries, the disease rate is of the order of 10-20% in the endemic regions and many of these patients have significant disability. It is therefore important that prevention and limitation of disability is incorporated as a matter of policy in the lymphatic filariasis elimination programmes. The care and the attention that the programme willprovideto patients with these disabilities will certainly enhance the quality of life of these patients. In addition, such a move will also enhance the acceptability of elimination programmes, since they will be catering to the needs of the affected people.

The following points may be made in this context:

Many patients suffer recurrent acute attacks of ADL because they do not undertake simple measures of hygiene and foot care. They also do not recognize minor lesions as a source of entry for bacteria and the consequent development of ADL attacks. Preventing ADL attacks is the best way to arrest the development of disability and even reversing the process in many cases.

Patients with long standing lymphoedema and multiple attacks of ADL require life long care for their disease.The health staff needs to understand the reasons why patients fail to follow the advice regarding hygiene and foot care. For this they must be trained to listen to, observe, train and encourage the patients.

Much can be done at the peripheral level itself and much can he achieved by way of disability prevention and limitation at very little financial cost per patient using commonly available things like soaps, creams etc. Of these, protective footwear is the most expensive item, but it is a relatively durable commodity. Here, it is important to make the best possible use of locally available resources and guide the patient to do likewise.

Effective prevention and limitation of disability requires the following:

Practice of simple measures of hygiene using soap and water.Early recognition and prompt treatment of entry lesions.

Referral of patients with lymphoedema who do not respond to treatment athome.

Referral of patients with hydrocoele for surgery.

It may not be practical to attempt and implement action to achieve all the above simultaneously. Priorities may need to be determined at national, regional and local levels to meet the problem step-by-step in a phased manner. In order to identify negative factors that hinder or slow down the disability prevention programme, feed-back information and incorporation of evaluation procedures are also necessary in this as in any other public health programme.

VECTOR CONTROL

Antilarval measures

Before carrying out antilarval measures, a map should be prepared showing all breeding places. The actual water surface area in each breeding site should be measured at least twice a year, once during the rainy season and once during the dry season.

Use of insecticides: As far as possible, breeding places should be cleared of scum and vegetation before the larvicide in applied, so as to maximize its efficiency. Larviciding is commonly used in urban areas, primarily against C. quinquefasciatus.

This species is generally resistant to organochlorine insecticides. However, newer selective insecticides in the organo-phosphorus, carbamate, and pyrethroid groups can provide effective control for this vector.

Commonly used larvicides are the organophosphorus insecticides such as temephos, fenthion, fenitrothion, chlorpyrifos, and pirimiphos-methyl. Temephos is relatively non-toxic to mammals and rapidly biodegradable; it can therefore be used in mosquito breeding sites that are also sources of drinking water. However, its residual effect is too short to be effective in polluted water. Chlorpyrifos is relatively toxic but it remains active in polluted water where C. quinquefasciatus normally breeds. Certain

insect development inhibitors, such as methoprene, have also been used for C. quinquefasciatus control.

The following dosages have been used against C. quinquefasciatus:

a) Chlorpyrifos: dosage 0.1-1.0 mg of active ingredient per litre of water; remains highly active for 12-24 weeks;
b) Methoprene: dosage 1.0 mg of active ingredient per litre of water inhibits emergence of adults for some 21 days.

New formulations of pyrethroids: Synthetic pyrethroids with long lasting residual effects (up to one year) can be highly successful in controlling adult mosquitoes when used for total indoor spraying in urban settings. Furthermore, new pellet formulations (soap with DEET and permethrin as active ingredients) have good efficacy against Mansonia adults and residual protection when applied on human skin. Among the household insecticide products, mosquito coils that contain knockdownsyntheticpyrethroids also give reasonably good protection against Culex and Mansonia mosquitoes.

BIOLOGICAL CONTROL

a) Biocides

Bacillus sphericus alternated with B. thuringenesis has been shown to be a viable approach.

b) Polystyrene beads

Control of mosquito vector breeding in closed water systems (pit latrines and cess pits) through use of expanded polystyrene beads has been extremely effective in certain urban areas with endemic filariasis.

Anti-adult Insecticides

The usefulness of insecticides in the control of filariasis is limited. As with malaria control, the selection of insecticide, dosage, and the scheduling of cycles and rounds should be developed from knowledge of vector bionomics, human habits, housing conditions, weather patterns, and the interrelationship of these variables with disease transmission by the vector. Indoor biters and indoor resters are most vulnerable to residual spraying. These include most of the anopheline vectors of filariasis.

Insecticide-impregnated bednets and curtains

Treatment of mosquito nets with a residual pyrethroid insecticide, such as permethrin or deltamethrin is helpful.

The net is placed in a plastic bag containing a dilute solution of insecticide. The exact quantity of liquid needed to treat the net, with negligible run-off, should be used. The dosage applied is about 0.5 g of active ingredient/m2. The treated net is allowed to dry and the residual effect of the insecticide can last for 6 months or more. Mosquitoes seeking a blood meal will come into contact with a lethal dose of insecticide while trying to pass through the net to feed on the person inside.

Community measures

The frequency of human-vector contact can be reduced by: the construction of better housing, with mosquito screens and/or mosquito-proof bedrooms; the use of mosquito nets, with or without insecticide impregnation, repellents, mosquito coils and proper clothing; the use of latrines; and the covering of breeding sites. In rural areas

cattle are often tethered in front of bedroom windows so as to attract the mosquitoes away from the human inhabitants.

Objectives of Morbidity Control

To educate both patients and health workers to intensify local hygiene, limb positioning and regular exercise can relieve suffering, prevent exacerbation and progression of symptoms, and even reverse some of the clinical damage already sustained.

To make disability prevention and alleviation, an essential component of programmes to eliminate lymphatic filariasis.

To ensure that all needy patients have access to quality health education and disability alleviation techniques.

Acute attacks

Attacks of Adenolymphangitis (ADL) play an important role in lymphatic filariasis. Although the disease produces gross deformities, it is these episodes of ADL that force patients with lymphatic filariasis to seek medical attention. Recurrent attacks of ADL also hasten the progression of filarial oedema resulting in elephantiasis (Pani et al., 1995). Surveys conducted in Pondicherry and Sherthallai estimated a frequency of 4.47 ADL episodes per year for bancroftianfilariasis and 2.2 episodes for Brugianfilariasis. On an average, such attacks last for four days, which result in abstinence from work and thus economic loss. In Sherthallai, the economic loss due to Brugianfilariasis was estimated to be 1.6 lakh mandays per year (Sabesan et al., 1992). It is therefore important to prevent and promptly treat these ADL attacks in patients with lymphatic filariasis.

Until recently several factors were reported to be the cause of ADL attacks. These included excreted or secreted parasite products (Ottesen, 1980) and exposure io fresh L3 infections (Partono, 1987). It is increasingly being recognised that secondary bacterial infections play an important role in the aetiology of these acute attacks. Studies in experimental animals and in patients with lymphatic filariasisOlszewski et al., 1994, Pani et al., 1995) have demonstrated these organisms in the skin and lymphatic fluid.

It has been previously shown that ADL attacks in patients with lymphatic filariasis are precipitated by secondary bacterial infection which gains entry through broken skin (Shenoy et al., 1995).

Patients with filariasis associated ADL have been treated with repeated courses of the widely available antifilarial drug DEC (Ottesen, 1985). Although the effectiveness of Ivermectin in the treatment of microfilaremia of bancroftian (Kumaraswami et al, 1988) and Brugianfilariasis (Shenoy et al., 1992) has been well established, the utility of the drug in the management of ADL has not been tested so far. The use of antibiotics and antinflammatoryagents for the management of ADL attacks has been anecdotal and not based on controlled clinical trials.

Apart from filarial oedema, the most important clinical manifestation of Brugianfilariasis is the episodes of adenolymphangitis. These episodes also have considerable socio-economic implications since they cause significant economic loss due to lost mandays. The economic loss due to Brugianfilariasis in Shertallai, which is situated in Alleppeydistrict, was estimated to be 1,60,000mandays/year (Sabesanetal, 1992).

The etiology of these episodes is unclear and the role of bacteria and parasites or parasite products in the production of these episodes has been examined by several workers (Ottesen, 1980, Partono, 1987). Previous studies have shown that ADL episodes are common in manual labourers who are accustomed to walking barefoot and who are also engaged in occupations that are likely to produce injuries to limbs (Partono, 1987). It is also recognised that damaged lymphatics (as they occur in lymphatic filariasis) are prone to invasion by streptococci (Ewert et ah, 1980).

Co-infection by Helminths and Mycobacteria

WHO estimated that there was about one third of the global population infected by TB, and in 2010, there were an estimated 8.8 million incident cases of TB globally, mostly occurring in Asia (59%) and Africa (26%) [WHO report 2011]. Meanwhile, in 2009 WHO also reported that there were an estimated 225 million malaria cases, mainly distributed in Africa (78%), South-East Asia (15%) and the Eastern Mediterranean (5%) [WHO report 2011]. In 2012, there were an estimated 436 million people at risk of *Schistosomiasishaematobium*infection in Sub-Saharan Africa, of which 112 million were infected, with an estimated 393 million people at risk of *Schistosomiasis mansoni* infection, of which 54 million were infected [WHO progress report 2001-2011]; an estimated 120 million people in tropical and subtropical areas of the world were infected with lymphatic filariasis in 2014 [WHO report 2015]. These figures suggest that there is an overlap of endemic regions between TB and parasitic disease, which may lead to co-infection of these diseases in the population.

Infection with tissue-invasive and/or intestinal helminths affects a large proportion of human populations living in tropical and subtropical regions. The 3 most commonly found geohelminths, Ascarislumbricoides, Trichuristrichiura, and Necator

sp., are each estimated to infect more than a billion humans worldwide (Bundy, 1994) and filariae and schistosomes together infect more than 350 million people. Generally, individuals living in endemic areas are infected with multiple helminth infections that are an important cause of morbidity, particularly in children (Bundy, 1994). Helminth infections have been associated with growth stunting (Stephenson, 1989; Nesheim, 1989), with poor cognitive performance in children (Nokes, 1991; Bundy, 1994), and with malabsorption (Sheehy, 1961; Nesheim, 1989; TheinHlaing, 1993).

The amelioration malaria severity is achieved by the combined induction of Th1 and Th2 immune responses with increased interleukin (IL)-5 and IFN-γ production [Dolo H et al 2012, Van der Werf N et al 2013]. On the other hand, pre-patent filariasis exacerbates malaria severity through immunosuppression of IFN-γ and initiation of activation of CD4 + CD25 + FoxP3+ T-regulatory cells [Tetsutani K et al 2009].

The investigation of the relationship between human helminth infections and the immune response to non-helminth antigens is of great public health significance for a number of reasons. If pre-existing infections can influence immune responses against unrelated antigens the implications for the effectiveness of vaccination programs, especially in developing countries, may be significant. Indeed, a recurring problem of vaccination campaigns in regions of the developing world has been the poor immunogenicity of vaccines (Edelman, 1987; Levine, 1993) including BCG, and vaccine failure even where coverage is high (Patriarca, 1991; Baily, 1980). One plausible explanation for this might be that such populations are generally infected with helminth parasites. The relevance of this concern is demonstrated by two recent studies, one in an area endemic for schistosomiasis (Sabin, 1996), and anotherendemicloronchocerciasis (Cooper, 1998), where helminth infection was been

shown to inhibit the development of type 1 (or Thl) responses to tetanus toxoid following tetanus vaccination. Evaluating the impact of parasitic disease infection on the efficacy of BCG vaccination against M. TB. Ferreira *et al.*[*Ferreira AP et al 2002,*] and Elias *et al.*[*Elias D et al 2005*] found that intestinal parasitic infections might significantly alter the protective immune response to BCG vaccination and/or polarize the general immune response to the Th2 profile since Th2-like interleukin-10 responses induced by intestinal parasites might interfere in the BCG-induced Th1-like interferon-γ response. Therefore, in areas of high prevalence of co-infection, anti-parasitic chemotherapy prior to immunization may greatly enhance the efficacy of BCG vaccination.

While there are now several examples of how immune imbalance created by a parasitic infection can affect subsequent responses to disparate immunological stimuli, it should be noted that, despite provocative reports (e.g. those implicating viral hepatitis in severe malaria (Thursz, 1996) and schistosomiasis (Pereira, 1995) there is little substantive evidence supporting a role for immune response bias promoted by one parasitic infection on susceptibility or resistance to other diseases. The rapid progression of AIDS in tropical and subtropical countries offers a pertinent example of a situation where parasitic infection might be an important co-factor in shaping the course of an unrelated disease (Bentwich, 1996). Indeed in vitro, HTV viral replication has been shown to be increased in cells from patients with filarial infections in whom there was a definite Th2 bias (unpublished data). Moreover, the failure of certain vaccines in tropical countries - such as BCG in South India (Baily, 1980) and oral cholera vaccine in South Americans of low socio-economic status (Suhayono, 1992) - shown to have efficacy in nontropical countries has suggested that concurrent helminth

infection may play an important role in altering the immune response to exogenously administered antigens.

There are a number of ways in which helminthiases may be a direct or contributive factor in poor immune responses to mycobacteria. First, infections with small intestine-dwelling helminths (A. lumbricoides and hookworm) have a particularly great impact in populations on marginal diets and have been linked to childhood malnutrition (Crompton, 1992) and xerophthalmia (Bhattacharya, 1982).

Malabsorption due to the presence of intestinal helminths is known to be associated with micronutrient and macronutrient deficiencies (Nesheim, 1989; TheinHlaing, 1993) and, as malnutrition and micronutrient deficiency are recognized as an important cause of acquired immunodeficiency (e.g., infants with severe protein-energy malnutrition are susceptible to mycobacterial infections), concurrent helminth infection may contribute to the increased risk of infection in malnourished children.

The immune response to pathogens has been investigated according to the Thl/Th2 T helper cell phenotype dichotomy of cytokine secretion: helminth infections are typically associated with predominantly Th2-type cytokines (e.g. IL-4, IL-5, and IL-10) (King, 1991), while protective immunity to intracellular organisms (e.g. intracellular bacteria, protozoa, and viruses) typically require a Th1 pattern of cytokine secretion (e.g. IL-2, IL-12, and IFN-y) (Del Prete, 1994). While these two sets of cytokines have been shown to exert counter-regulatory effects on each other, the Thl-Th2 dichotomy, at least in humans, probably reflects a mixed cytokine response in which one or the other phenotype is predominant (Kelso, 1995). A hyporesponsive cellular immune response to parasite antigens, characterized by decreased cellular proliferation and decreased production of IFN-y and IL-2, is common in many parasitic

diseases such as filariasis and schistosomiasis. The reasons for this appear to be multifactorial and include regulation by IL-10, duration and intensity of infection, in utero exposure to parasite antigens and the presence of soluble suppressive parasite products. Responses to non-parasite antigens such as PPD however, appear to remain intact (as measured by IFN-y production) in patients with filariasis (Sartono, 1996), although several studies have documented a poorresponse to non-parasite antigens in onchocerciasis (Steel, 1991; Soboslay, 1992) and schistosomiasis (Sabin, 1996). For example, a diminished cellular response to tetanus toxoid is seen in subjects with onchocerciasis compared to uninfected controls (Prost, 1983; Kilian, 1989; Cooper, 1998).

Infections caused by intestinal helminths is very common in south India. One study (Kang, 1998) estimated the prevalence of hookworm infections to be 62% in an area near Vellore, South India while the prevalence of Strongyloides and Ascaris was lower (15.4 and 6.4% respectively). In a similar socio-economic area near Madras, South India, the prevalence of exposure to mycobacterial antigens, as measured by reactivity to purified protein derivative (PPD) in the general population is estimated to be virtually 100% to PPD-B and about 60-70% to PPD-S by age 24. Profound effect of Strongyloidiasis infection on the systemic cytokine responses in ATB and LTB and indicate that coincident helminth infections might influence pathogenesis of TB infection and disease. (George PJ et al 2015)

There is evidence from animal models that the presence of one intestinal nematode parasite influences the immune response to another (Curry, 1995). The cytokine milieu at the time of antigen presentation is known to influence the pathway, which a subsequent immune response follows (Del Prete, 1994), and there is evidence

that other helminth infections may push an immune response to bacterial and viral infections towards a Th2 phenotype (Pearlman, 1993; Actor, 1993; Curry. 1995; Sabin, 1996). This may have important consequences for the type of immune response generated in the presence of geo-helminth infection.

Aims and Objectives

AIM AND OBJECTIVES

- To study the clinical management of patients with various forms of lymphatic filariasis and therapeutic aspects of lymphatic filariasis

- To study the underlying immunological mechanisms that are the basis of the immunomodulatory effects of filarial infection on the immune system in individuals with filariasis alone are who have mycobacterial co-infections.

Clinical Management and Therapeutic Aspects of Lymphedema

CLINICAL MANAGEMENT OF LYMPHEDEMA

Acute inflammatory episodes (acute attacks)

The aetiology of acute inflammatory episodes in lymphatic filariasis has long been a subject of debate and confusion. Indeed, a variety of terms have been used in the literature to describe them, including 'adenolymphangitis (ADL)', 'acute attack', 'filarial attack', and 'endemic lymphangitis', among others [Dreyer G et al]. As early as the 1920s, some scientists argued that bacterial infections were the primary cause of 'filarial' lymphangitis [Grace A et al]. In 1924, the British Filariasis Commission went so far as to state that "all the pathological manifestations" of lymphatic filariasis were caused by secondary bacterial infections [Anderson J et al]. During World War II, clinical and pathologic studies of soldiers with adenolymphangitis and other early clinical manifestations demonstrated the importance of Wuchereria bancrofti adult worms or 4th-stage larvae [Wartman et al]. The debate continued after World War II, when the role of the immune system in triggering adenolymphangitis, as well as other forms of filarial pathology, was emphasized [Ottesen et al].

One of the major factors contributing both to the debate and the confusion during the latter half of the 20th century was the relative lack of emphasis on careful clinical observation and case definitions. In 1999, Gerusa Dreyer and colleagues, working in Brazil, defined two distinct clinical syndromes: acute filarial lymphangitis (AFL), caused by death of the adult worm, and acute dermatolym- phangioadenitis (ADLA), associated with secondary bacterial infection [Dreyer G et al]. AFL is characterized by lymphangitis that progresses distally or in a 'retrograde' fashion along the lymphatic vessel, producing a palpable 'cord'. Rarely, AFL is accompanied by mild fever, headache, and malaise. Distal lymphoedema may occur, but is usually mild and reversible, i.e. self-limited. In contrast, ADLA (a term first used by Olszewski)

[Olszewski et al] develops in a reticular or circumferential pattern, and is clinically similar to erysipelas or cellulitis. Symptoms of local pain and swelling, as well as fever and chills, are present. In filariasis-endemic areas, ADLA occurs much more commonly than AFL [Dreyer G et al].

Although there is general agreement on the two clinical syndromes as described by Dreyer et al., it has also been suggested that exposure to 3rd-stage filarial larvae causeslymphangitis and triggers the onset or progression of lymphoedema. A role for 3rd or 4th-stage larvae in lymphangitis or lymphoedema is supported by animal studies, experimental infections [Nutman TB et al], reports of disease in individ- ual patients travelling from non-endemic areas [Moore et al], and epidemiologic observations that associate incidence of acute adenolymphangitis with filarial transmission intensity [Shi ZJ et al, Bockarie et al]. However, a case definition has not been established for larva-associated lymphangitis that distinguishes it from AFL or ADLA; this makes epidemiological study difficult. Additional work is needed to clarify the incidence, possible mechanisms, and clinical expression of larva-associated filarial lymphangitis and to assess its public health importance in filariasis-endemic areas.

Recent speculation also has focused on a potential role for Wolbachia in the pathogenesis of filaria-related disease [Taylor et al]. Lammie and colleagues have suggested that the pathogenesis of disease in lymphatic filariasis is multifactorial, and have proposed a model that involves the immune system and also allows for a variety of possible causes [Lammie PJ et al].

Limited attention has been paid to the differences in pathogenesis and clinical manifestations between brugian and bancroftian filariasis. Obvious differences have been noted, such as the absence of male urogenital involvement and chyluria and the

much more frequent occur- rence of abscesses at the site of lymph nodes in brugian filariasis. However, the reasons for these differences are poorly understood.

Acute dermatolymphangioadenitis

Pathogenesis

Evidence for a bacterial aetiology of ADLA in filariasis- endemic areas comes from the distinctive clinical signs and symptoms, isolation of bacteria at the time of the acute episode, and changes in antibody titres between acute and convalescent serum specimens [Pani S et al, Olszewski et al, Shenoy RK et al, Suma et al]. In India, the bacteria most frequently associated with ADLA are Group A Streptococcus. Other bacteria are often found in cultures, including those that are usually regarded as non-pathogenic [Olszewski et al, Vijayalakshmi N et al].

Available evidence indicates that the immune system may amplify or modulate ADLA. The relative infrequency with which bacteria are isolated from patients with ADLA [Olszewski et al, Vijayalakshmi N et al], as well as from persons with cellulitis in areas not endemic for lymphatic filariasis [Duvanel et al], suggests a role for inflammatory mediators [Dupuy et al], perhaps even in the absence of bacteria.

Little has been published on the antimicrobial sensitivity of bacteria isolated from persons with ADLA in filariasis- endemic areas. Available experience suggests that theorganisms most commonly involved are sensitive to penicillin; thus, penicillin is usually recommended for treat- ment [Dreyer G et al].

Clinical descriptions of ADLA in filariasis-endemic areas are remarkably similar to those of erysipelas and cellulitis, about which much has been written in the dermatologic literature [Bonnetblanc et al]. Group A Streptococcus is the classical

causa- tive organism for erysipelas, and lymphoedema is a well recognized risk factor for erysipelas and cellulitis in areas not endemic for lymphatic filariasis [Dupuy et al].

Economic and psychosocial impact

Cost Studies from India, Ghana and Haiti indicate that ADLA treatment costs to patients range from US$ 0.25 to US$ 1.62 per episode, as much as two days' wages [44,45,60- 63]. In Sri Lanka, Chandrasena reported costs of US$ 7.38 per episode for care from private practitioners, although most patients received free treatment at government clinics [Chandrasena et al]. These costs included direct costs of treatment, including self-medication, as well as travel. Two studies also included costs of food and accommodation [Gyapong et al]. In all cases, except for consultations with herbalists in Haiti,patientsseekingcarefromhealthcentresorprivate providers spent more money than those seeking care from traditional practitioners, primarily because these providers had higher consultation charges. In addition, payment was often provided in-kind when care was given by members of the extended family or traditional practitioners. At the upper end of the spectrum, Kron et al. calculated costs for personal expenses in the Philippines as high as US$ 25 per ADLA episode, excluding lost wages [Kron et al].

Productivity

Much of the burden of ADLA comes not from treatment costs, but from indirect costs due to lost productivity. ADLA episodes significantly affect patients' abilities to carry out both economic (farming, market activities, building) and domestic (household chores, cooking, taking care of children) activities [Gasarasi et al].ADLA episodes are more disabling than other febrile ill- nesses [Gayapong J et al]. This incapacitation results in productivity losses; studies in India and Tanzania showed that patients with ADLA spent an average of 2.7–3.6 hours less per day on economic activities than

controls [Ramaiah KD et al, Nanda B et al].

Studies indicate that ADLA episodes reduced potential community labour supply in Ghana by 0.79% [61] and in Indian communities by approximately 0.1% [Ramaiah KD et al, Nanda B et al]. While these figures represent a much smaller loss than that from chronic filarial disease (7% of potential labour lost), they do not adequately capture the impact of ADLA at the level of the household. Household-level effects, including time lost from work and school for caregivers, have not been studied in detail.

Even with these modest estimates, the productivity lost due to ADLA represents a significant loss of potential income. Sabesan estimated that US$ 160000 per year is lost to ADLA among persons with lymphatic filariasis in Pondicherry, India [Sebasean et al], while other studies in India estimate a national figure of US$ 60–85 million lost per year [Ramaiah KD et al]. Kron estimated that US$ 38 million is lost annually due to ADLA in the Philippines [Kron et al].

Quality of life

In recent study, patients in India ranked ADLA higherthan lymphedema andhydrocelein-termsof severity, with an average severity score of 25–27 on a scale of 0–28. Patients also cited 'very severe problems' in the domains of mobility, self-care, usual activities, pain, anxiety/depression and social participation on an extended EuroQol scaling system [Kumari KA et al]. They reported curtailing their activities and interactions with others in an attempt to prevent future ADLA attacks from occurring. Other studies have noted the pain, restrictions and dependency that result from ALDA episodes, but have not translated this into standard quality-of-life indicators [Coereil et al, Suma TK et al].

Treatment and Prevention

Treatment

Treatment recommendations for ADLA include rest, cooling the affected area to relieve pain and limit thermal- related damage to the skin, analgesics and antipyretics to relieve pain and fever, systemic antibiotics, and elevation of the affected limb [Shenoy RK et al, Dreyer G et al]. Little is known about the degree to which antibiotics shorten the duration of ADLA episodes, but as with erysipelas and cellulitis in areas not endemic for lymphatic filariasis [Bonnetbanc et al], antibiotic treatment is recommended [Dreyer G et al].

Prevention

Basic lymphoedema management

An increasing number of studies have documented the effectiveness of basic lym- phoedema management, as recommended by WHO, in reducing the incidence of ADLA episodes [Addiss et al, McPHerson et al, Kerketta et al]. In Guyana, McPherson found that 10 of 11 patients had reported ADLA during the six months preceding enrolment in a hygiene education programme, compared to none of them during the six months after enrolment [McPHerson et al]. A recent evaluation by WHO reported dramatic reductions in incidence of ADLA in Sri Lanka, Zanzibar (United Republic of Tanzania), and Madagascar [WHO report]. In India, several placebo-controlled studies have observed significant decreases in ADLA incidence among lymphoedema patients who only received instruction in foot care [Shenoy RK et al].

Reductions in ADLA frequency can be maintained for several years through home-based care. In Haiti, the reported incidence of ADLA during the year before beginning treatment was 2.1 episodes per year; this decreased to 0.6 episodes after

hygiene and skin care were emphasized [Addiss et al]. A follow-up assessment 18 months after the patients 'graduated' from clinic visits, but continued lymphoedema care at home, showed an annual incidence of 0.5 ADLA episodes per year [Dahl BA et al]. Suma and colleagues reported sustained practice of self-care among patients in an area endemic for brugian filariasis; some two years after patients had received 'foot care' education, 95.3% reported having fewer or less severe ADLA episodes, with a mean incidence of 2.8 acute attacks per year [Suma TK et al].

Prophylactic antibiotics.

For patients who continue to experience frequent episodes of ADLA despite basic measures of hygiene and skin care, prophylactic antibiotics are recommended [Dreyer G et al]. This practice is also recommended in non-endemic countries for patients with lymphoedema who have recurrent cellulitis. The effectiveness of prophylactic antibiotics has been evaluated in several studies. Olszewski examined the effect of benzathine penicillin, given at three-week intervals for one year, on the incidence of ADLA, and reported a dramatic decrease, with recurrent episodes occurring only in 9% of patients [Olszewski et al]. In a placebo-controlled trial in Vellore, India, lym- phoedema patients who received prophylactic penicillin experienced greater decreases in ADLA incidence than those who only received training in foot care [Joseph A et al]. How- ever, in similar studies in Kerala, India, Shenoy and colleagues found that, for most patients, antibiotics provided little additional benefit if foot care was regularly practiced [Shenoy RK et al]. Kerketta and colleagues, in Orissa, India, observed lower rates of ADLA among patients who were randomized to receive foot care and penicillin prophylaxis than among patients not receiving penicillin, although the difference was not statistically significant [Kerketta AS et al]. A recent Cochrane review concluded that although penicillin and foot care

appear to reduce the frequency of cellulitis, further studies are needed to document the effectiveness of these measures [Badger C et al].

Antibiotic soap

An unpublished study from Haiti found that the incidence of ADLA in lymphoedema patients decreased to a similar extent (from 1.1 episodes to 0.4 episodes per year) in patients who washed with antimicro- bial soap and those who received standard soap [Addiss DG et al], suggesting that hygiene itself was more important than the antimicrobial content of the soap.

Participation in patient support groups.

Participation in patient support groups has been shown to decrease the number of ADLA episodes and improve quality of life among lymphoedema patients in Haiti [Coreil J et al].

Risk of death.

Fatal outcomes for ADLA are thought to be uncommon, but most programme managers and clinicians who care for patients with lymphoedema are aware of at least a few cases in which ADLA progressed to septicaemia and death. The actual incidence of fatal outcomes with ADLA is unknown, and risk factors for severe or fatal ADLA are poorly characterized. The clinical experience of Dreyer and others indicates that elderly patients, alcoholics, and patients with malnutrition, hypertension, diabe- tes, or chronic cardiac or pulmonary disease may be at increased risk of severe ADLA [Dreyer G et al].

Acute filarial lymphangitis

Pathogenesis

As noted above, among persons born and raised in areas endemic for bancroftian filariasis, episodes of AFL, due to death of the adult worm or 4th-stage larva, are less severe and have less systemic involvement than ADLA. Systemic involvement may be greater in 'immune-naïve' immigrants to endemic areas. Classical AFL was described extensively in European soldiers and US during World War II [Wartman W et al]. AFL is commonly observed following individual or mass treatment with DEC [Sutanto et al], and this is considered evidence of the drug's macrofilaricidal efficacy [Dreyer et al].

Treatment

Treatment of AFL is supportive. Cold compresses, rest, and analgesics are recommended. Treatment with anti- filarial drugs during acute inflammatory episodes used to be recommended, but now is not considered indicated [Shenoy et al, Dreyer et al].

Acute filarial lymphangitis and clinical disease

The degree to which AFL triggers or hastens the development of hydrocele in bancroftian filariasis has been investigated by several authors. Norões and colleagues reported a 22% incidence of acute hydrocele following a single 'scrotal nodule event', whether spontaneous or induced by DEC [Noroes et al]. Overall, 5% of men with scrotal nodules (adult worm death) developed hydrocele that persisted for 18 months or longer. Similar findings were observed in Haiti following mass treatment with DEC and albendazole [Addiss DG et al]. Hussein and colleagues in Egypt found that 14 of 16 infected men developed detectable fluid in the tunica vaginalis cavity after treatment

with DEC and albendazole, of whom three developed chronic hydrocele [Hussein et al]. It is unclear whether the lifetime risk of acute or chronic hydrocele is increased by DEC treatment, or whether the drug merely synchronizes adult worm death and, therefore, resulting hydrocele.

AFL appears to trigger the onset of lymphoedema less frequently than it does hydrocele, and persistent lymphoedema following AFL is unusual in the absence of other co-factors [Dreyer G et al].

METHODS

MORBIDITY CONTROL

A broad outline of the strategy and management tasks involved in implementing a disability prevention programme within the lymphatic filariasis programme is given below. In addition a community based approach for rehabilitation of patients is also outlined.

TASKS AT CENTRAL HEALTH AGENCY LEVEL

- Adopt disability prevention in lymphatic filariasis patients as a policy
- Plan national implementation of the policy of disability prevention.
- Identify agencies to carry out implementation
- Establish priorities, determine timing, phasing and extent of coverage
- Budget allocation and fund mobilization
- Incorporate disability parameters in the information system (records, returns)
- Plan appropriate task-oriented, competency based training programmes
- Provide for interdepartmental collaboration where necessary

TASKS AT REGIONAL / STATE LEVEL

- Plan regional implementation of national policy and programme.
- Specify responsibilities and tasks for all staff involved.
- Arrange appropriate task-oriented staff training.
- Ensure provision of supplies - budget, purchase, inventory, distribution.
- Ensure access of patients to referral facilities.
- Ensure that patients who have hydrocoele and other genital problems have access to surgical care at the local hospitals.

- Plan and implement appropriate supervision, evaluation and encouragement procedures.

TASKS AT LOCAL/CLINIC LEVEL

Ensure that the clinic staff are competent to carry out the following tasks:

- Plan clinic implementation of regional disability control policy.
- Complete nationally agreed disability records and returns to an acceptable and agreed standard of performance.
- Ensure provision of materials necessary for disability control - indent, purchase, inventory.
- Identify disability preventive actions needed for individual patients and ensure that these actions are taken.
- Allocate specific responsibilities and tasks to all the clinic staff and train staff in these tasks.
- Ensure that individual patients are given practical training in appropriate self-care procedures until they can correctly demonstrate them and actively encourage the patients to continue to practice these procedures thereafter.
- Identify local sources and special needs of protective footwear.
- Identify local sources of other supplies (water, soap, medicated creams).
- Implement appropriate supervision and evaluation procedures.
- Ensure that patients are familiar with support facilities for any problem relating to their disability.

LIST OF SPECIFIC TASKS TO BE CARRIED OUT AT VARIOUS LEVELS

TASKS AT HOME LEVEL

To be carried out by the patient and helped and encouraged by family members and the health worker.

Objective 1 : Practice hygiene and foot care

Action 1 : Wash the leg at least once a day.

Dry between the toes and folds using a small cloth

Pat wet the leg with clean water at room temperature

Begin soaping at the highest point and wash down the leg towards the foot

Use a small cloth to clean between the toes

Rinse with clean water and repeat until water runs clear

Wash the other leg even if it looks normal

Pat the area with a clean towel. Do not rub.

Careful attention to be given to the nails. Trim nails if necessary.

Resources : Knowledge

Water

Soap

Clean cloth, towel

Action 2 : Taking care of entry lesions

Look for entry lesions between the toes and in the deep skin folds

		Wash with potassium permanganate solution and place cloth soaked in potassium permanganate in deep skin folds and in between the toes
		Use the medicated cream prescribed by the doctor / health worker
Resources	:	Knowledge
		Potassium permanganate solution
		Medicated cream
Action 3	:	Elevate the leg
		Keep the leg elevated while sitting whenever possible
		Elevate the entire leg at night
Resources	:	Knowledge
Action 4	:	Exercise the leg
		Practice simple exercises taught by the health worker
Resources	:	Knowledge
Action 5	:	Protect the legs from injury using footwear Wear shoes
		that are loose and comfortable
Objective 2	**:**	**Prevent occurrence of ADL attacks**
Action	:	Inspect daily the affected leg for cracks and injuries.
		Carefully separate the toes and look for evidence of infection or injury.
		Recognise wounds and cracks early through daily inspection.
		Report to health worker if entry lesions have not healed in a few days or if it worsens (as indicated by increasing redness, swelling or pus).

Practice exercises daily.

Use protective footwear

Keep footwear in good condition and replace them promptly when they are worn out.

Resources : Soap

Antiseptic

Medicated cream

Special footwear

Objective 3 : Recognise and report onset of ADL attacks

Action : Report to health worker

Pain and swelling of limb

Lymphadenitis

Associated with fever, myalgia, vomiting.

Resources : Knowledge

Objective 4 : Recognise and manage ADL attacks

Action 1 : Relieve the pain

Apply a cloth compress soaked with water (the cooler the better).

The compress should go all round the leg.

Change the compress when it becomes warm

Cool the leg until the pain goes away

Rest and elevate the leg comfortably as much as possible

Do not exercise during an acute attack

Drink plenty of water

Take paracetamol tablets prescribed by the doctor/health worker

Action 2 : Hygiene and treatment of entry lesions

Wash the leg with soap and water, but more gently and more carefully than usual

Dry the leg more gendy and carefully than usual

Identify and care for entry lesions

Apply the prescribed antiseptic to the skin

Apply medicated cream to entry lesions

Action 3 : Report to the Health worker / Doctor if there is

High fever with shaking chills

Headache, drowsiness, confusion

Fever or pain that does not subside within 24 hours of treatment

Resources : Knowledge

Paracetamol

Antiseptic

Medicated cream

TASKS AT CLINIC LEVEL

To be carried out by health worker.To recognize, instruct, support and monitor patients with lymphoedema

Objective 1 : Promote the practice of hygiene and foot care by patients with lymphoedema

Teach the patients the proper way to wash their legs

Observe patients washing their legs at home

Pay attention to the nails. Trim nails if necessary.

Look for entry lesions between the toes and in the deep skinfolds

Wash with potassium permanganate solution and place cloth soaked in potassium permanganate in deep skin folds and in between the toes

Apply medicated cream prescribed by the doctor

Teach patient the correct method of elevating the leg

Teach patients the simple exercises

Inspect footwear and encourage patients to wear protective footwear

Resources : Manual

Potassium permanganate solution

Medicated cream

Objective 2 : Prevent acute attacks in patients with lymphoedema

Action : Identify high-risk patients.

Make a special note in the record.

Make a baseline record of location and extent of lymphoedema, precipitating factors for ADL

Teach high-risk patients to look for and report any entry lesions and acute attack

Assess high-risk patients periodically for change in lymphoedema

Reinforce the principles of foot care and hygiene

Resources	:	Manual
		Supplies of medicated creams, antiseptic
Objective 3	**:**	**Recognise and treat acute attacks**
Action 1	:	Relieve the pain
		Relieve the pain by using cold compresses
		Elevate the leg comfortably as much as possible
		Advise patient not to exercise during an acute attack
		Advise patients to drink plenty of water
		Provide paracetamol tablets
Action 2	:	Treat entry lesions
		Wash the leg with soap and water, but more gently and more carefully than usual
		Dry the leg more gently and carefully than usual
		Identify and care for entry lesions
		Apply the prescribed antiseptic to the skin
		Apply medicated cream to entry lesions
		Provide sufficient antiseptic and medicated creams
Action 3	:	Report to the doctor if there is
		High fever with shaking chills
		Headache, drowsiness, confusion
		Fever or pain that does not subside within 24 hours of treatment
		Entry lesions have not healed in a few days or if it worsens (as indicated by increasing redness, swelling or pus).

Resources : Manual

Paracetamol

Antiseptic

Medicated cream

COMMUNITY BASED REHABILITATION OF LYMPHOEDEMA PATIENTS AT CLINIC LEVEL

General objectives

1. To arrest the process of disability
2. To rehabilitate individuals already dehabilitated.

Sub-objectives

Objective 1 : Identify and categorize cases in need of help

Action : 1. Review all registered cases by interview and clinical examination and collect the following data:

(a) Disease type

(b) Clinical activity and disease status

(c) Physical disability

(d) Age

(e) Nature of existing problems

a. Social relationships

b. Changes in economic status

(f) Education and marketable skills

Objective 2: All cases found to be in need of rehabilitation identified, categorised and given appropriate priority rating.

Resources : Clinical records

Psychological/social/economic status assessment forms

Trained staff familiar with local milieu

Time for staff to perform duties

Rehabilitation need categorization and priority scale.

Objective 2 : Action instituted to arrest the process of rehabilitation.

Action : Teach self-care to the patient and monitor his or her self-care activities

Help patient to obtain suitable aids for self-care (including protective footwear) and daily living

Counsel "family" group regarding giving appropriate support to the patient.

Resources :

1. Manual for self-care
2. Manual for patient and family counselling
3. Staff training
4. Time
5. Protective footwear (unless it is available through local resources).

Objective 3 : Links developed with local resources.

Action : Identify, list and review all local resource groups, e.g., health education, sports, training, medical, non-governmental agencies, employers, religious and social groups, professional group shoemakers.

Establish contact with local resource groups

Link patient in need of help with specific local resources

Develop on-going information exchange with local resource groups

Resources : 1. Training in community relations

2. Local credibility of health worker

3. Time

Objective 4 : Clinic support of action instituted by the family

Action : 1. Make a list of families to be visited and supported on aregular basis

2. Regularly visit - to encourage family's efforts, to find out any problems and to work out solutions

3. Record kind of support needed and actions taken

Resources : 1. Knowledge and aptitude

2. Time

Objective 5 : Action to be taken for patients likely to benefit from referral to rehabilitation services

Action : Select cases for referral (only after community resources areexhausted)

Write referral letters

Arrange referrals

Follow up referrals

Resources : Trained staff with:

a) knowledge of referral services (manual and local list of

services)

b) ability to use criteria for referral and make appropriate referral decisions.

AT HOME LEVEL

Objective 1 : Disabled person fully accepted as a member of the family.

Family action : Provide opportunity for the disabled person to perform activities and accept responsibilities normal for his or her age and position.

Disabled person's action: Accepts and carries out responsibilities and fully utilizes the opportunities provided by the family.

Resources : Counselling from health / rehabilitation worker

Support from the community

Information sheet

A handbook to reinforce the teaching given

Objective 2 : Disabled person encouraged and assisted, if necessary, to practise daily self-care.

Family action : Work with the disabled person as he or she practises self-care activities.

Disabled person's action; Practices self-care activities.

Resources : Knowledge of required self-care activities.

Manual of self-care

Knowledge of disease, symptoms and treatment

May need financial assistance for protective footwear

Objective 3 : Disabled person encouraged to function within the community in the most normal way possible.

Family action : Encourage disabled person to undertake social activities normal for age and sex, e.g. play with other children, go to school, attend social functions.

Interact with members of the community to provide opportunities for disabled person to participate in normal activities.

Work with community leaders and health professionals to change negative attitudes of community members.

Resources : Knowledge of disease

Positive attitude

Support from health /rehabilitation worker

Credibility and standing of health / rehabilitation worker

AT COMMUNITY LEVEL

Objective 1 : Disabled person fully accepted as member of the community

Community Action

- Community leaders imparl relevant information about
- What disabled persons can do and their problems (environmental and social barriers to normal lile activities)
- The causes and effects of specific types of disabilities commonly found in the community
- The care and help that can be given at home and by the community.
- Community leaders act as advocates for disabled person's interests through

legislature, pressure groups and mass media.

- Draw attention to what disabled persons can do to help themselves, e.g., "show" cases, sports.
- Community leaders set personal examples of acceptance of the disabled person.

Resources : Technical information from clinic and health system

Mass media

Special skills/talents of community members

Funds raised within the community

Objective 2 : Local community resources identified and made accessible to disabled persons.

Action by community leaders and "pressure groups"

Assist clinic staff to identify local resources

Assist clinic to build referral network through tapping key people in community having time, connections and money andassist disabled person to recognize and take advantage ofopportunities

Resources : Information from clinic

Manual

Interested people in the community

Outside "catalysts"

Objective 3 : Responsibility accepted for the development of additional resources for disabled persons.

Action : By community leaders and support groups.

Explore possible ways to assist the disabled, e.g. local

employment, cheaper locally made protective aids (includingprotective footwear), adult education, income generatingactivities.

Raise funds from within the community

Scout for outside funding to develop resources in the community

Resources : Time, money, ingenuity

Information from national and regional levels

Local organization

Manual

Objective 4 : Disabled persons encouraged to participate in decision making activities of the community.

Action by community leaders and support groups.

Identity disabled persons already established in community

Invite disabled person to participate in community committees, e.g. in health developmental, social, administrative activities

Encourage development of local associations to work for disabled persons.

Make available to the local association new and relevant information.

Strengthen the confidence of disabled persons and their families by encouraging their efforts.

Resources : Manual

Local people with interest and drive

Outside leadership

STUDY POPULATION

This study was carried out in three panchayats consisting a population of more than 5,000 in Poondi Panchayat Union of Tiruvellore Taluk with an intense geohelminth infections and active filarial infection of 10-25%. The sample size was estimated about 5200 consisting of both males and females, in the age group 6 to 65 year old group.

All individuals were registered as per the epidemiology unit work procedures and the information will be recorded on individual card. Individuals aged 6-65 years were eligible for blood collection as well as stool examination. Disease status and information on passing of worms was collected by census taker during registration. If the individual was absent during census, this information was collected at the centre, where all eligible persons are directed for blood collection using ICT Diagnostic Kit as per the instruction given in the kit. Group and individual number were recorded on the cover of the kit at the time of blood collection with sketch pen. After the blood collection at the centre, a container was issued to each asking them for a sample of stool on the same day or overnight. The group and individual number were recorded on the cap as well as on the sticker pasted on the container using magic marker before issuing the container to the individual for stool collection. The overnight specimen was collected on the next day by the morning team.

On an average, 80-100 blood and stool specimens were collected in a day.

Patients admitted to the study were chosen from among those attending the Filarial clinic located in an area endemic for Brugianfilariasis. The protocol required all patients admitted to the study give their informed consent to participate in the study.

Both men and women, in whom pregnancy has been excluded, were eligible to be included in the study.

All patients admitted to the study had underlying filarial oedema with a history of at least two attacks of adenolymphangitis (ADL) in the past year. An attack of ADL was defined as one in which there was pain, tenderness, lymphangitis with or without lymphadenitis along with local warmth in the affected limb. Fever, oedema and other constitutional symptoms were also recognised as part of the syndrome of acute attacks. Each attack was graded for its severity and duration based on a previously tested out protocol.

However patients who had acute cellulitis due to an identifiable cause in the absence of underlying lymphoedema (injuries, fungal infections), thrombophlebitis, history or physical examination suggestive of sexually transmitted disease were not considered for admission.

Patients with all grades of lymphoedema were admitted to the study. We used a modification of previously defined criteria (WHO, 1992) to determine the grade of lymphoedema for each patient.

For the purpose of this study, the lymphoedema was classified as follows:

Grade 0	:	No oedema
Grade I	:	Oedema reversible on elevation of the affected limb
Grade II	:	Persistent oedema, no skin changes
Grade III	:	Persistent oedema with thickening of skin
Grade IV	:	Oedema with thickening of skin and warty, nodular excrescences.

Study design

This was a double-blind placebo-controlled study of multiple of oral penicillin, DEC or local care of the affected limb in 150 patient with lymphoedema of the limbs due to filariasis and who have had at leasttwo episodes of adenolymphangitis (ADL) in the past year.

Information regarding the use of footwear by the patient and their occupation was recorded before their admission to the study.

All patients enrolled were initially hospitalized for four days. On day one adetailed examination of the patient including measure of the limbs and laboratory investigations were carried out. All patients admitted to the study were then initiated to a programme of cleaning of the affected limb. This programmeconsisted of cleaning the affected limb every night with soap and water, keeping theaffected limb dry, clipping the nails and applying salicylic acid ointment to webs of the toes, nails and sides of the feet every night.

Patients were then allocated to a yearly treatment with one of the five treatment regimens on the basis of random sampling.

1. Oral penicillin group

Patients in this group received two tablets - one containing 800,000 units of penicillin G potassium and the other containing placebo, every day.

2. DEC group

Patients in this group received two tablets - one containing DEC 50 mg (approximately 1 mg/kg) and the other containing placebo, every day.

3. DEC plus antibiotic group

Patients in this group received two tablets - one containing DEC (50 mg) and the other containing 800,000 units of penicillin G potassium every day.

4. Local antibiotic ointment group

Patients in this group received two tablets of placebo every day and they applied framycetin cream locally to the affected limb whenever they had a local infection or injury, during the one year period.

5. Placebo group

This group received two tablets of placebo every day for one year.

Since we used a double blind design for the study, blinding was achieved by using look- alike tablets of penicillin, DEC and placebo. Similarly, to blind the local antibiotic group, all the other groups also received a bland zinc oxide cream in similar looking containers.

From 2nd to 4th day the trial drug was administered orally according to the random allocation schedule. Each patient received two identical tablets contained in a packet meant for a days supply. They were observed for any adverse effect of the drug and were properly trained in local care of the affected limb.

On discharge from the hospital on the 4th day they were given 15 days supply of the trial drug, salicylic acid ointment, framycetin/placebo ointment and soap solution for washing the affected limbs. Patients in all drug groups were instructed to apply this framycetin/placebo ointment locally to the affected limb whenever they had a local infection or injury. Compliance was assessed by using standard methods such as surprise field checks and pill counts done at assigned intervals by the medical social

workers. They were also instructed to come to the hospital every two weeks for examination and to replenish the medicines or whenever they had an ADL attack.

Follow up

Each patient enrolled in the study was seen every two weeks by members of the research team and was questioned and examined for the occurrence of an ADL attack. The assessment included a grading of the various components of ADL and a quantitative index was assigned to each episode. At each attendance the same examinations were conducted and efficacy was assessed by evaluating the number of episodes of ADL and their duration. Every attack of ADL was confirmed by the physician/health worker.

All ADL episodes which occurred during the treatment period were treated with oral antibiotics (combination of sulfamethoxazole 800 mg and trimethoprim 160 mg bid) and paracetamol 500 mg tid x 5 days. When the local infection in the affected limb did not clear with the application of the blinded antibiotic/zinc oxide cream, patients were given povidone iodine ointment to be applied locally.

During the second year, when the treatment phase was completed, none of the patients received any drugs but were instructed to continue their foot care programme as advised at the beginning of the study. They were also instructed to come to the hospital every two weeks for examination and any ADL episodes occurring during this follow up period were also assessed and treated as mentioned above.

Since all the drugs used in the study are already extensively used with proven safety, no special safety measurements were made. However, patients were questioned for sensitivity to penicillin and trial drug tablets were given only after excluding

sensitivity. Drugs were administered initially only by medical or nursing staff who are trained in resuscitation procedures, as sensitivity reactions are most likely to occur during initial doses.

All statistical comparisons were made using the student's t-test or test of correlation.

RESULTS

A total of 175 patients were screened for admission to the study. However 25 patients could not be included in the study for a variety of reasons: follow up not assured (12), penicillin allergy (5), presence of chronic illnesses (7) or on prolonged DEC (1) as mentioned in **Table.1**.

TABLE 1

Reasons for exclusion from the study

	Reason
1	Age < 18 years
2	Insufficient no of ADL attacks
3	ADL secondary to obvious injury
4	Associated cardiovascular diseases
5	Diabetes mellitus
6	Epilepsy
7	Bronchial asthma
8	Prolonged antibiotic treatment
9	Follow up not assured
10	Psychiatric illness
11	Carcinoma cervix
12	On DEC treatment

Among the 150 cases included in the study, there were 69 males and 81 females. Their clinical presentations on admission are shown in **Table 2.**

TABLE 2

Clinical presentation on admission

	Clinical features	No
1	Fever, pain, tenderness and local warmth	75
2	Lymphangitis alone	1
3	Lymphadenitis alone	13
4	Lymphangitis and lymphadenitis	54
5	Lymphadenitis and cellulitis	4
6	Abscesses with other symptoms	3

Their age range was 18 to 67 years (median 43). The number of patients in each drug group and their age and sex distribution are shown in **Table 3.**

The total No. of ADL episodes in the 150 patients in the year prior to admission were 684 and their occurrence in a patient ranged from 2 to 20. While 52 patients had only two episodes, there were three patients with grade IV oedema who had 20 ADL attacks in the pre-treatment year.

The filarial oedema involved the lower limbs in 147 patients out of the 150 enrolled for the study; either one leg alone in 82, both the legs in 52 and both upper and lower limbs in 13 patients. In the remaining three patients one upper limb alone was affected. The duration of the lymphoedema ranged from two months to 50 years

(median 17 yrs.). While the duration was less than one year in six patients, it was more than ten years in 104 patients.

Among the study subjects, 81 had lymphangitis. Only in 31 patients there was centrifugal spread of the lymphangitis. Even in this group 30 had a local lesion in the affected limb, responsible for precipitation of the ADL through secondary bacterial infection

Eighty seven of the 150 patients used footwear regularly while 52 used it irregularly. Eleven patients with gross lymphoedema (Grade III and IV) did not use any footwear at all due to the deformity of the oedematous limbs.

All these patients were admitted to the study only after the resolution of any associated ADL episode. A precipitating cause likely to produce an ADL could be identified in the affected limb in 81 patients**.** While the commonest of these was moniliasis in the webs of the toes (69 patients) other causes included chronic paronychia (5), pyoderma (4), eczema (2) and fissures of the feet (1).

TABLE 3

Comparison of ADL attacks before and after Treatment

SI. No.	Age	Sex	Pre. DEC Tt.	ADL Pre Tt.	ADL 1st Yr (Tt.)	ADL 2nd Yr.
1	35	M	No	2	1	1
2	49	F	Yes	4	1	2
3	39	M	Yes	2	5	0
4	46	M	Yes	4	1	0
5	61	F	Yes	5	1	1
6	52	F	Yes	3	2	2
7	18	F	Yes	6	1	2
8	33	M	Yes	8	1	0

SI. No.	Age	Sex	Pre. DEC Tt.	ADL Pre Tt.	ADL 1st Yr (Tt.)	ADL 2nd Yr.
9	53	M	Yes	2	0	1
10	50	F	Yes	2	0	2
11	48	F	Yes	2	0	0
12	35	M	Yes	5	2	2
13	32	M	Yes	3	3	5
14	65	M	Yes	10	1	1
15	35	F	Yes	**3**	1	1
16	27	M	Yes	**2**	3	1
17	50	M	Yes	**5**	1	2
18	52	F	Yes	10	0	3
19	40	M	Yes	5	1	0
20	35	M	Yes	3	1	0
21	38	F	Yes	2	0	1
22	40	F	Yes	3	2	0
23	25	M	Yes	2	7	0
24	40	M	Yes	3	3	0
25	50	F	Yes	2	1	0
26	49	F	No	2	0	0
27	25	M	Yes	2	2	0
28	37	F	Yes	4	1	0
29	20	F	Yes	3	0	1
30	42	F	Yes	5	1	1
31	42	M	Yes	4	1	1
32	48	M	Yes	4	1	0
33	40	F	Yes	3	4	0
34	38	M	Yes	3	2	1
35	43	M	Yes	4	2	1
36	65	M	Yes	5	2	0
37	25	M	Yes	2	1	0
38	55	F	Yes	4	1	0
39	52	F	Yes	4	1	0
40	38	M	Yes	4	7	2
41	40	F	Yes	6	1	1

SI. No.	Age	Sex	Pre. DEC Tt.	ADL Pre Tt.	ADL 1st Yr (Tt.)	ADL 2nd Yr.
42	38	F	Yes	2	0	0
43	21	M	Yes	2	0	0
44	24	M	Yes	2	0	0
45	42	M	Yes	3	0	0
46	53	M	Yes	2	0	0
47	25	F	No	3	1	0
48	49	F	Yes	4	0	0
49	40	M	Yes	3	0	0
50	19	M	Yes	2	r 0	0
51	33	F	Yes	2	0	0
52	40	F	Yes	2	0	0
53	40	M	Yes	2	0	0
54	35	M	Yes	2	0	0
55	48	F	Yes	2	2	0
56	56	M	Yes	3	1	0
57	60	M	Yes	2	1	0
58	20	M	Yes	2	0	0
59	62	M	Yes	3	0	0
60	57	F	Yes	5	3	0
61	33	F	Yes	2	0	0
62	32	M	Yes	6	1	0
63	35	M	Yes	2	3	0
64	23	M	Yes	2	0	0
65	63	F	Yes	2	0	0
66	47	F	No	3	1	0
67	19	M	No	3	1	0
68	55	F	No	3	2	0
69	38	F	No	5	0	0
70	44	M	No	2	0	0
71	49	F	Yes	3	0	0
72	40	M	No	4	1	0
73	24	M	Yes	3	0	0

SI. No.	Age	Sex	Pre. DEC Tt.	ADL Pre Tt.	ADL 1st Yr (Tt.)	ADL 2nd Yr.
74	62	M	Yes	5	2	0
75	18	M	No	2	0	0
76	32	M	No	3	0	0
77	39	F	Yes	3	0	0
78	47	M	Yes	3	1	0
79	35	F	Yes	5	2	0
80	47	M	Yes	5	2	0
81	58	M	Yes	10	0	0
82	33	M	Yes	3	2	0
83	38	F	Yes	3	1	0
84	29	M	Yes	2	0	0
85	20	M	Yes	5	0	0
86	45	M	Yes	5	5	0
87	45	F	Yes	2	0	0
88	32	M	Yes	3	1	0
89	38	M	Yes	4	0	0
90	33	M	Yes	3	0	0
91	33	F	Yes	2	0	0
92	34	M	Yes	4	1	0
93	38	F	Yes	6	0	0
94	48	F	Yes	2	2	0
95	30	M	Yes	2	0	0
96	35	M	Yes	4	0	0
97	30	F	Yes	2	2	0
98	40	M	Yes	3	0	0
99	62	M	Yes	10	0	0
100	24	F	Yes	2	0	0
101	54	F	Yes	6	1	0
102	45	F	Yes	10	1	0
103	33	F	Yes	4	0	0
104	31	M	Yes	10	0	0
105	32	F	Yes	3	0	0

SI. No.	Age	Sex	Pre. DEC Tt.	ADL Pre Tt.	ADL 1st Yr (Tt.)	ADL 2nd Yr.
106	27	M	Yes	6	0	0
107	23	M	Yes	4	1	0
108	35	M	Yes	2	1	0
109	54	F	Yes	3	0	0
110	40	M	Yes	5	0	0
111	40	M	Yes	5	1	0
112	68	F	Yes	12	2	0
113	47	M	Yes	2	0	0
114	60	M	Yes	5	0	0
115	39	F	Yes	12	1	0
116	21	F	Yes	6	0	0
117	32	M	Yes	5	1	0
118	45	F	Yes	3	0	0
119	61	M	Yes	8	0	0
120	50	F	Yes	2	0	0

The mean number (±) SE of ADL attacks in the pre treatment period were as follows: penicillin 4.6 ± 0.8 , DEC 4.0 ± 0.5, penicillin + DEC 5.8 ± 0.9, local antibiotic 4.2 ± 0.5 and placebo 4.7 ± 0.7. The difference between the groups was not significant ($p > 0.3$ for all comparisons). A total of 355 ADL episodes were observed in 103 patients (73.57%) during the two year study period: 127 during the first year and 228 during the second year .

Among those who had recurrence, 57 had the episodes during treatment phase, 85 had during the follow up phase and out of them 39 patients had ADL during both years. Of these only in 37 instances an entry lesion for bacteria could not be located in the affected limb (**Table 4**). In the remaining 318 episodes the precipitating lesions

observed in the affected limb were minor injury (128), moniliasis (101), pyoderma (70), eczema (16), fissure foot (2) and insect bite (1).

TABLE 4

Organisms recovered from sites of infection

	Organism	No
1	Staphylococcus aureus	9
2	Streptococcus pyogenes	4
3	Coagulase negative staphylococci	1
4	Mixed bacterial flora	16
5	Bacteria +Fungus	2
6	No organism	1

The reduction in the number of ADL attacks in the treatment year was significantly less in the placebo group when compared with the penicillin group ($p < 0.012$), DEC group ($p < 0.042$) and penicillin + DEC group ($p < 0.001$). In all except the placebo group there was an increase in the number of ADL attacks in the year following treatment when compared to the year of treatment. However this increase was statistically significant only for the two penicillin groups: penicillin ($p < 0.007$) and penicillin + DEC ($p < 0.001$).

In the placebo group the number of ADL attacks continued to decline in the follow up year as well but this decrease was not statistically significant.

When the infection in the affected limb was not cleared by the local application of blinded antibiotic/placebo ointment, patients were given povidone iodine ointment for local application on 68 occasions. Thus it was used in the placebo group during 29 occasions, local antibiotic group 15, oral penicillin group 11, DEC group 9 and in penicillin + DEC group 4 occasions.

Comparisons regarding the number of ADL attacks were also made based on the grades of lymphoedema. There was a significant correlation between the grade of oedema and the number of pre-treatment ADL attacks (r = 0.989). The No. of ADL attacks are represented in **Table 5.**

TABLE 5

Number of ADL attacks in the previous year

	Attacks	**No**
1	2 - 5 attacks	74
	Gr. I edema	11
	Gr II edema	17
	Gr III edema	42
	No edema	4
2	> 5 attacks	7
	Gr. I edema	2
	Gr II edema	3
	Gr III edema	2

There was a significant reduction in the number of ADL attacks in all grades of oedema ($p < 0.008$ for all comparisons) between the pre-treatment period and the treatment phase. As in the case of the analysis done according to drug groups there was an increase in the number of ADL attacks in the follow up phase when compared to the treatment year. This was however statistically significant only for patient with grade III ($p<0.025$) and grade IV ($p<0.009$) oedema.

The changes in the grades of oedema although were not analysed statistically, it is obvious that little or no improvement was seen in patients with grade III or grade IV edema.

Surgical intervention

The goal of lymphedema therapy is to restore function, reduce physical and psychologic suffering, and prevent the development of infection.

Initiate therapy for lymphedema as early as possible before extensive, irreversible fibro sclerotic changes occur in the interstitium. Strict compliance with treatment techniques is essential, even though they are often cumbersome, uncomfortable, inconvenient, and time-consuming, with treatment lasting throughout the lifetime of the individual. The majority of compliant patients can be treated successfully with conservative measures.[Schmitz KH et al, Mayrovitz HN et al, Pereira De Godoy JM et al]. In secondary lymphedema, the underlying etiology (ie, neoplasm, infection) should also be properly treated, in order to relieve the lymphatic obstruction.

Surgery

Surgical treatment is palliative, not curative, and it does not obviate the need for continued medical therapy. Moreover, it is rarely indicated as the primary treatment modality. Rather, surgical treatment is reserved for patients who do not improve with conservative measures or for cases in which the extremity is so large that it impairs daily activities and prevents successful conservative management.

A myriad of surgical procedures have been advocated for the treatment of lymphedema, reflecting a lack of clear superiority of one procedure over the others. Multiple physiologic and excisional techniques have been described. None of the physiologic techniques has clearly documented long-term favorable results; further evaluation is necessary. Moreover, many of the physiologic techniques also include an excisional component, making it difficult to distinguish between the 2 approaches.

Physiologic and Excisional Surgery

As previously mentioned, surgical treatment is palliative, not curative, and it does not obviate the need for continued medical therapy. Moreover, it is rarely indicated as the primary treatment modality but is instead reserved for patients who do not improve with conservative measures or for cases in which the extremity is so large that it impairs daily activities and prevents successful conservative management.[Boyd J et al]

In general, surgical procedures are classified as physiologic or excisional. However, many physiologic techniques include an excisional component, making it difficult to distinguish between the 2 approaches.[Warren AG et al]

Physiologic surgery

Physiologic procedures attempt to improve lymphatic drainage. Multiple techniques have been described, including omental transposition, buried dermal flaps, enteromesenteric bridging, lymphangioplasty, and microvascular lympholymphatic anastomosis.[Narushima M et al] None of these techniques has clearly documented favorable long-term results. Further evaluation is necessary.

Rarely, venous-lymphatic anastomosis is performed in patients with severe lymphedema and a functioning venous system. Reports in the literature suggest that this procedure is effective only in cases of secondary lymphedema. Prophylactic lymphovenous anastomosis has been performed in patients undergoing extensive pelvic lymph node dissection who have a high risk of developing lymphedema.

Excisional surgery

Excisional techniques remove the affected tissues, thus reducing the

lymphedema-related load. Some authors advocate suction-assisted removal of subcutaneous tissues, but this technique is difficult because of the extensive subcutaneous fibrosis that is present. Additionally, this approach does not reduce the skin envelope, and the lymphedema often rapidly recurs. Suction-assisted removal of subcutaneous tissue followed by excision of the excess skin envelope has no clear advantage over direct excisional techniques alone.

The Charles procedure is another quite radical excisional technique. This procedure involves the total excision of all skin and subcutaneous tissue from the affected extremity. The underlying fascia is then grafted, using the skin that has been excised. This technique is extreme and is reserved for only the most severe cases. Complications include ulceration, hyperkeratosis, keloid formation, hyperpigmentation, weeping dermatitis, and severe cosmetic deformity.

A variant of the Charles procedure, total superficial lymphangiectomy, involves debulking of the entire limb. Van der Walt et al developed a modified Charles procedure in which negative-pressure dressing was employed following debulking surgery, with skin grafting delayed for 5-7 days.[Van der Walt et al] In a report on 8 patients suffering from severe primary lymphedema who underwent the procedure, the authors reported that the patients experienced no major complications. Minor complications, including operative blood loss and, in 3 patients, the need for additional grafting, did occur.

Staged excision has become the option of choice for many authors. This procedure involves removing only a portion of skin and subcutaneous tissue, followed by primary closure. After approximately 3 months, the procedure is repeated on a different area of the extremity. This procedure is safe and reliable and demonstrates the

most consistent improvement with the lowest incidence of complications.

Maggot debridement therapy for elephantiasis nostrasverrucosa is effective, and, owing to increasing antimicrobial resistance, is gaining popularity. It can be used in conjunction with tangential surgical debridement. Hyperammonemia due to secretions with from maggots can occur. [Borst GM et al]

Preoperative details

Prior to surgery, appropriate documentation is necessary to evaluate the outcome of treatment. This includes photographic documentation as well as extremity measurements. Ideally, these measurements are of limb volume by water displacement, although some rely on circumferential measurements alone. Obtain measurements and photographs at the same time of day each time, document affected extremities and contralateral extremities, and preferably conduct documentation in the morning after extremity elevation in bed overnight.

Institute strict elevation and pneumatic compression, if available, 24-72 hours prior to surgery. This allows maximum excision to be performed. The extremity must also be free of infection at the time of surgery; a single dose of preoperative intravenous antibiotic is administered.

Intraoperative Details

Surgery for removal of lymphedematous tissue includes the following steps:

- After the establishment of appropriate anesthesia, the operative field is sterilized and draped according to surgeon preference
- A pneumatic tourniquet is placed at the root of the extremity and insufflated after the extremity has been exsanguinated

- A longitudinal incision is made along the entire extremity, and skin flaps, 1.0-1.5 cm thick, are elevated
- Subcutaneous tissue is then excised, with care taken not to injure peripheral sensory nerves
- Some authors also excise a strip of deep fascia; however, this should not be performed around joints, because it may cause instability
- Once the subcutaneous excision is complete, redundant skin is resected; often, a strip that is 5-10 cm wide may be removed
- The wound is closed over suction drains

Postoperative care

Postoperatively, the extremity is immobilized in a splint and elevated; the patient is placed on strict bed rest.

Antibiotics may be continued until drain removal, according to surgeon preference. Drains are typically removed at 5-7 days postoperatively, as dictated by a decrease in drain output. Sutures are removed at 10-14 days and replaced by Steri-Strips.

The patient should be measured for a new compression garment when the new dimensions of the extremity have stabilized. After approximately 10 days, the patient may gradually begin dependency on the extremity with compression bandages or an elastic garment in place.

Postoperative follow-up

Once discharged from the hospital, the patient should be seen regularly in the outpatient clinic. Patients must wear compression garments continuously for 4-6 weeks;

dependency on the involved extremity may be gradually increased at the discretion of the treating physician.

Once he or she has healed to physician satisfaction, the patient may return to a normal routine of elevation at night and compression garment therapy during the day.

Follow-up visits should include documentation of circumferential measurement or water displacement of the affected and contralateral extremities, as well as photographic documentation.

When staging procedures, allow approximately 3 months between procedures to allow complete healing of the initial operative site.

Multi modality treatment for the reversal of Grade IV lymphedema- a case study

Here we present, a case study of 37 year old female, admitted with giant lymphedema (Grade IV) for 27 year duration (Fig. 1). Preoperative lymphoscintigraphy picture revealed multiple lymphatic channels, with huge dermal backflow and multiple inguinal lymph nodes. (Fig. 2). For which multi modality treatment and treated successfully with an aesthetically and functionally acceptable lower limb. This case was unique for its size and presentation of the right lower limb. After getting initial basic foot care in the form of daily cleaning with Betadine Scrub, antifungal powder together with foot elevation with a course of oral penicillin (800mg/twice daily for one week), the patient was taken for Manual Lymph Drainage (MLD) and multi layer bandaging for ten days. This was done twice daily with respiratory physiotherapy and walking.

Earlier experience from our surgical intervention towards grade I and grade II for its reversal had empowered us to include this surgical correction as our choice in the

multimodality treatment. (B.-B Lee et al., Lymphedema.2011). Hence we performed a new technique of superficial excision of alternate lumps in three stages at the interval of 6 weeks. Then we carried out Nodovenal shunt to retain the achieved results and avoid recurrence of this swelling. This multi modality treatment for this patient has given the best outcome in cosmetic and functional result (Fig. 3).

This patient was followed for the past ten years without any recurrences or complications. Following this we started and recommend multimodality treatment in grade III and IV lymphedema patient as a regular practice of morbidity control of lymphatic filariasis. This case is presented here for its uniqueness in terms of presentation and the outcome we have achieved using multimodality treatment in lymphatic filariasis.

Fig.1. Giant lymphedema (Grade IV -Right leg)

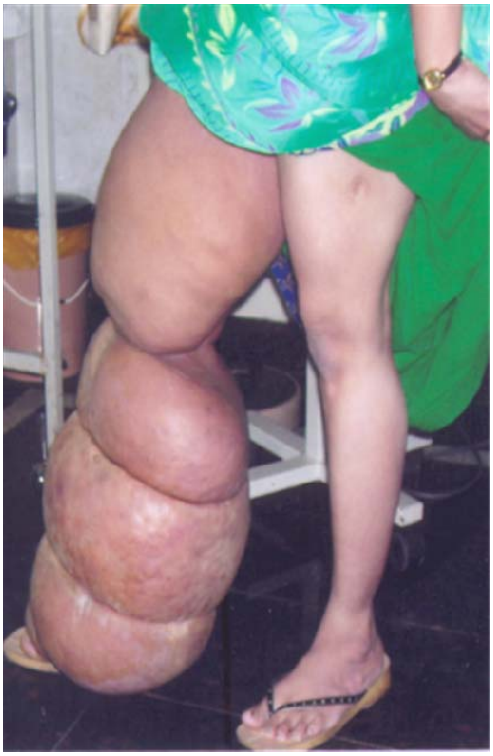


Fig. 2. Pre operative lymphoscintigraphy picture showing multiple lymphatic channels, with huge dermal backflow at the later pictures and multiple inguinal lymph nodes.

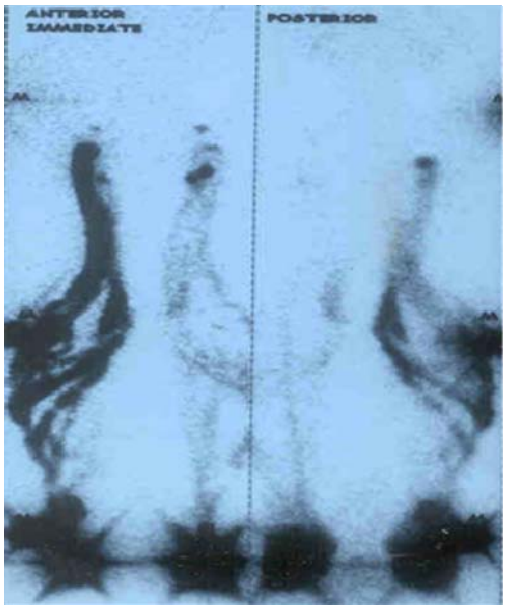
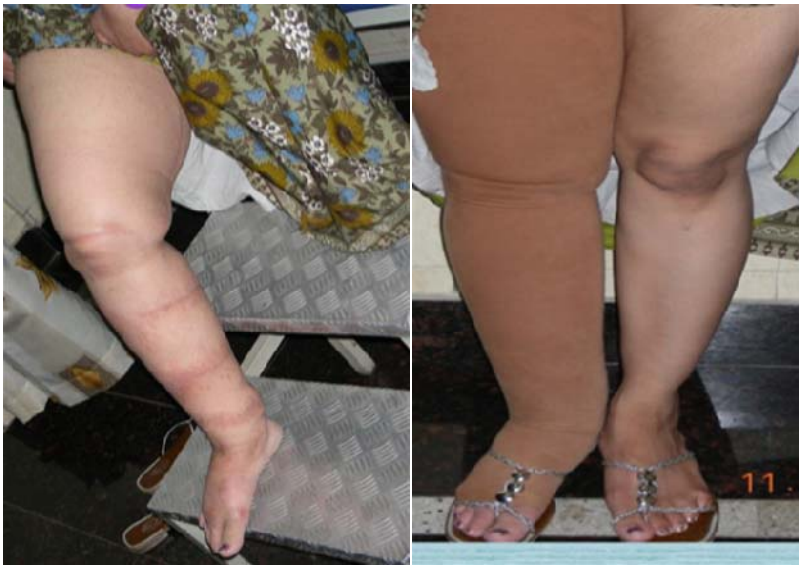


Fig. 3. Reversed Grade IV lymphedema, after multi modality treatment (Manual Lymph Drainage, Multilayer bandaging and Surgical Intervention (Nodovenal Shunt).



Immunological Studies for Co-infection and Lymphatic Filariasis

IMMUNOLOGICAL STUDIES FOR CO-INFECTION AND LYMPHATIC FILARIASIS

INTRODUCTION

TLRs are innate immune receptors commonly associated with the initiation of inflammatory processes (Kawai et al). Upon binding to their cognate ligands, TLRs activate the innate immune response, leading to the production of proinflammatory cytokines and chemokines (Iwasaki A et al). Studies of animal models of filarial infection and in vitro studies in humans have suggested that Wolbachia-derived molecules from filarial parasites are key inducers of proinflammatory cytokines (Hiese et al, Taylor et al). Moreover, this inflammatory response to Wolbachia has been shown to be mediated primarily through TLR2, TLR4, and TLR6. In addition, with a mouse model of onchocerciasis, a related filarial infection, it was demonstrated that Wolbachia interaction with the host innate immune system resulted in development of inflammatory keratitis, a characteristic feature of human onchocercal eye disease (Saint Andre et al). Downregulation of TLR on antigen-presenting cells (APCs) and T cells has been shown to be a possible mechanism by which deleterious pathology in clinically asymptomatic filarial infections can be circumvented (Semanani et al). Indeed, TLR downregulation, both in terms of expression and function, on monocytes and B and T cells appears to be a characteristic feature in patent filarial infections (Babu S et al). In addition, exposure of human dendritic cells to live filarial parasites has been shown to downregulate the expression and function of TLR3 and TLR4 (24). Finally, depletion of Wolbachia by doxycycline treatment has been associated with reduction in lymphatic pathology in filariainfected individuals (Debrah et al). We have previously shown that enhanced TLR expression is an important feature of chronic lymphatic pathology, with patients with lymphedema exhibiting significantly enhanced

expression of TLR2, -4, -7, and -9 mRNA in comparison to asymptomatic, infected patients (Babu S et al); however, the functional role of this differential TLR expression on immune responses engendered in CP individuals has not been studied. Hence, we wanted to examine whether the increased expression of TLRs would lead to increased expression of proinflammatory cytokines, which, in turn, could potentially promote pathology development. Moreover, activation of TLRs triggers a series of signaling events leading to activation of the NF-B pathway and, as a result, induction of inflammatory cytokines (Takeda et al). Therefore, we also examined the signaling cascade triggered by the activation of TLRs in filaria-infected and -uninfected individuals. Our study demonstrates that patients with chronic lymphatic pathology (CP)—in contrast to infected patients with subclinical pathology (INF) or uninfected, endemic-normal (EN) individuals—are characterized by an augmented production of proinflammatory cytokines, including Th1 and Th17 cytokines in response to the stimulation of three TLRs: TLR2, -7, and -9.

Study population.

We studied a group of 14 patients with filarial lymphedema (CP), 14 asymptomatic infected (INF) patients, and 14 uninfected endemicnormal individuals (EN) in an area endemic for lymphatic filariasis in Tamil Nadu, South India . CP patients were negative for circulating filarial Ag by both the ICT filarial Ag test (Binax, Portland, ME) and the TropBio Og4C3 ELISA (TropBio Pty. Ltd., Townsville, Queensland, Australia), indicating a lack of current active infection. They had undergone treatment with repeated doses of diethylcarbamazine. INF patients tested positive by both the ICT filarial Ag test and the TropBio Og4C3 ELISA as well as by BmA-specific IgG4 and IgG enzyme-linked immunosorbent assays (ELISA). EN patients were negative for circulating filarial Ag by both tests. BmA-specific IgG4 and

IgG ELISA were performed exactly as described previously (Lal RB et al). The three groups did not differ significantly in baseline hematological and immunological parameters, including total white blood cell count. All individuals were examined as part of a clinical protocol approved by Institutional Review Boards of both the National Institute of Allergy and Infectious Diseases and the Tuberculosis Research Center, and informed written consent was obtained from all participants

Characteristics of Study Population

	EN (n=15)	**INF (n=15)**
Median age, yr (range)	40 (20-59)	47 (19-65)
Gender (no. of subjects M/F)	7/8	6/9
ICT Card Test	Negative	Positive
W. bancrofti circulating antigen levels U/ml (median)	8 (3.45–28.87)	2,059.073 (136.52–33,125.87)

Methedology:

Isolation of PBMC and in vitro culture

Peripheral blood mononuclear cells (PBMCs) were isolated as described previously (5). PBMCs were cultured in 24-well tissue culture plates (Corning Incorporated, Corning, NY) at concentrations of 5 10^6 /well for 24 h. For phosphorylation assays, PBMCs were cultured with TLR ligands for 30 min, following which cell lysate was prepared using the phosphoprotein cell lysis kit (Bio-Rad, Hercules, CA) according to the manufacturer's protocol. The protein concentration was assayed using the Bio-Rad DC protein assay kit. For NF-kB estimation, PBMCs were cultured with TLR ligands for 3 h, and nuclear extracts were prepared using a nuclear

extraction kit (Panomics, Milan, Italy) according to the manufacturer's protocol. Cytokine ELISA. Levels of cytokines in the culture supernatants were measured using the Bioplex multiplex cytokine assay system (Bio-Rad). The cytokines analyzed were gamma interferon (IFN-□), tumor necrosis factor alpha (TNF-□), interleukin-17A (IL-17A), IL-23, IL-12p70, IL-1□, and IL-6. Net cytokine levels were calculated by subtracting cytokine levels in the medium control for each stimulated condition.

RNA preparation

RNA was isolated from PBMCs following culture with TLR ligands for 24 h. PBMCs were lysed using the reagents of a commercial kit (QIAshredder; Qiagen, Valencia, CA). Total RNA was extracted according to the manufacturer's protocol (RNeasyminikit; Qiagen), and RNA was dissolved in 50 ml of RNase-free water. cDNA synthesis. RNA (1 g) was used to generate cDNA using TaqMan reverse transcription reagents according to the manufacturer's protocol (Applied Biosystems, Fullerton, CA). Briefly, random hexamers were used to prime RNA samples for reverse transcription using MultiScribe reverse transcriptase.

Real-time RT-PCR

Real-time quantitative reverse transcription (RT)-PCR was performed in an ABI 7500 sequence detection system (Applied Biosystems) using TaqMan assays-on-demand reagents for MyD88, TRAM, TRIF, TIRAP, TRAF6, and an endogenous 18s rRNAcontrol. Relative transcripts were determined according to the manufacturer's protocol.

RESULTS

TLR2, -7, and -9 ligands induce significantly elevated proinflammatory cytokine production in filarial pathology. As previously published, the expression of TLR2, -4, -7, and -9 mRNA was significantly higher in CP compared to INF patients at baseline (Lal RB et al). To examine whether the increased expression of TLRs translates to increased function in terms of production of proinflammatory cytokines in lymphatic filariasis, we stimulated PBMCs from CP, INF, and EN individuals with TLR ligands and measured the levels of IFN-g, TNF-a, IL-17, IL-23, IL-12, IL-1b, and IL-6 following 24 h of stimulation that revealed several salient features. First, baseline as well as poly(IC) (TLR3), flagellin (TLR5), FSL-1 (TLR2/6), and single-stranded RNA (ssRNA) (TLR8) stimulated levels of proinflammatory cytokines were found to be not significantly different among the three groups (data not shown). Second, the TLR2 ligands Pam3Cys and HKLM induced significantly increased production of IFN-γ, TNF-α, IL-12, IL-1α, and IL-6 but not IL-17 and IL-23 in CP compared with both INF and EN individuals. Third, the TLR4 ligand LPS induced only moderately increased expression of TNF-α and IL-6 in CP patients compared with INF but not with EN patients. Fourth, TLR7 ligand imiquimod induced significantly increased production of IFN-γ, TNF-α, IL-17, IL-23, and IL-6 in CP individuals compared with INF individuals alone. Finally, TLR9 ligand ODN induced significantly increased production of IFN-γ, TNF-α, IL-12, IL-1α, and IL-6 as well as IL-17 and IL-23 in CP individuals compared with both INF and EN individuals. Thus, TLR ligands (especially, Pam3Cys, HKLM, LPS, imiquimod, and ODN) induce a very complex pattern of cytokine induction that is significantly more pronounced in CP patients compared with the other two groups. TLR ligand stimulation does not alter TLR adaptor gene expression in filarial pathology. Because TLRs are known to recruit

specific adaptor molecules to initiate downstream signaling and adaptor molecule expression is known to be controlled at the transcriptional level (O'Neill et al), we measured the mRNA levels of the five major adaptor molecules for TLR signaling: MyD88, TRAM, TRIF, TIRAP, and TRAF6. We failed to observe any significant difference in the expression levels of all of the above adaptors either at baseline or following stimulation with TLR ligands among the three groups. Our data therefore suggest that differential production of pro-inflammatory cytokines is not associated with a significantly altered transcriptional signature of TLR adaptors.

Stimulations	CP
Pam3cys	TNF-a IFN-g IL-1b IL-6 IL-12
HKLM	TNF-a IFN-g IL-1b IL-6 IL-12
LPS	TNF-a IL-6
Immiquinod	TNF-a IFN-g IL-17 IL-23 IL-6
ODN	TNF-a IFN-g IL-17 IL-23 IL-1b IL-12

DISCUSSION

Lymphatic filariasis is a disease characterized by a broad spectrum of clinical manifestations seen among the majority of infected people (Nutman et al). A subset of individuals with this infection has demonstrable pathology characterized, most notably, by lymphedema, hydrocele, and elephantiasis. The events that lead to the development of lymphatic pathology in filariasis are not fully understood, although the immune responses of the host to the parasite products are believed to play a significant role in determining development of pathology (Pfarr et al). TLRs are important initiators of immune responses through their ability to recognize a variety of microbial products (Takeda et al). TLR-dependent proinflammatory cascades triggered by microbial products must be tightly regulated to avoid severe pathology (O'Neill et al). In terms of clinical filarial disease, it is thought that this regulation is defective, and the resultant proinflammatory cytokines, in turn, are hypothesized to directly or indirectly (through downstream effects on various angiogenic and lymphangiogenic factors) lead to perturbations in the maintenance and function of the lymphatic endothelial system, resulting in a variety of complications, including lymphatic dilatation and lymphedema (Pfarr et al). Our findings demonstrate that proinflammatory cytokines are indeed elevated in CP individuals compared with INF or EN individuals in response to specific TLR ligands. Interestingly, the only TLRs that mediate this differential cytokine response are those that have been shown previously to have enhanced expression in CP individuals: TLR2, TLR4, TLR7, and TLR9 (Lal R.B et al). This suggests that (i) TLR-mediated release of proinflammatory cytokines is associated with the development of pathology, and (ii) this response clearly demarcates differences between those with overt, clinical pathology and those with asymptomatic or subclinical infection. While a part of the cytokine response to TLR stimulation is clearly blunted in asymptomatically

infected individuals, even compared to uninfected individuals, the significantly elevated response seen with CP over not only INF but also EN individuals suggests that heightened proinflammatory cytokine production is a characteristic feature of patients with lymphatic pathology. Even with the proinflammatory responses induced by TLR stimulation in CP individuals, however, distinct patterns of cytokine secretion can be observed. Thus, while TLR2 stimulation resulted predominantly in increased levels of Th1-type proinflammatory cytokines—IFN-, TNF-, and IL-12— TLR7 and -9 ligands also induced elevated levels of Th17-type proinflammatory cytokines IL-17 and IL-23. While TLRs are known inducers of other proinflammatory cytokines, very little is known about the role of TLRs in inducing type 17 cytokines (Marta et al). Our data suggest that TLR7 and -9 stimulation leads to IL-17 and IL-23 production and that this specific Th17 response is amplified in the setting of filarial pathology. TLR2 and TLR4 activation on T cells has been shown to induce IL-17 production in mice (Reynolds et al). To our knowledge, this is the first report describing the production of IL-17 directly in response to TLR stimulation in human PBMCs. In addition to TLR-mediated cytokine production, we examined TLR adaptor usage in filarial pathology. Signaling by all TLRs originates from the conserved Toll–IL-1R domain (O'Neill et al) and recruitment of the common adaptor molecule MyD88 leads to interaction and activation of the IL-1R-associated kinase family members, and subsequent activation of TRAF-6 (TNFR-associated factor-6), results in NF-B activation through the IB complex (19). Other Toll–IL-1R domain containing adaptor molecules include TIRAP/Mal, TRIF, and TRAM (O'Neill et al). These adaptors mediate TLR signaling either alone or in combination with MyD88 and confer the specificity of TLR-mediated inflammatory responses. Recently, it has been shown that MyD88 and TIRAP are the primary adaptor molecules recruited during TLR stimulation by filarial products (Hise et al) and that

MyD88 gene expression was significantly decreased in dendritic cells exposed to live filarial parasites (Semanani et al). We examined the gene expression of the above-mentioned TLR adaptors but did not observe any significant difference in the expression of MyD88, TRAM, TRIF, TIRAP, or TRAF-6 at the mRNA level in response to TLR stimulation between the three groups. This suggests that transcriptional regulation of TLR adaptors is not a primary mechanism in the differential cytokine response to TLR stimulation in filarial patients, although regulation at the posttranscriptional level would need to be examined as well to exclude any role for the TLR adaptors (Reviewd by Rajasekaran et al).

Helminth and TB Co- infection Immunological Responses

INTRODUCTION

Lymphatic filariasis is a disease that afflicts over 120 million people worldwide. The parasites that cause the infection (*Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*) are long lived and often induce asymptomatic (or subclinical) infections due, in large part, to the parasites' ability to manipulate the host immune system and to restrict inflammatory pathology [Mathers et al]. Modulation of the host immune response involves a variety of strategies, including induction of regulatory networks and dysregulation of innate and adaptive immune responses [Maizels et al]. Chronic filarial infections are associated with diminished expression and function of Toll-like receptors (TLRs) on antigen-presenting cells (APCs) and T cells [Babu S et al]. While the immune modulation associated with systemic filarial infections is primarily parasite-antigen specific, some bystander effects on routine vaccinations, allergic processes, and autoimmune diseases have been noted [Van Riet et al]. We have shown recently that filarial infection coincident with *Mycobacterium tuberculosis* (Mtb) infection significantly diminishes Mtb-specific Th1 (IL-12/IFN-γ) and Th17 (IL-23/IL-17) responses related to increased CTLA-4 and PD-1 expression (manuscript in press).

Mtb infects ~2 billion people worldwide, with 90% of Mtb-infected individuals having latent infection. TLR signaling has been postulated to play an important role in the host resistance to Mtb [Korbel DS et al]. The control of tuberculosis (TB) requires clearly delineated Th1 responses (IL-12, IFN-γ, and TNF-α) and, to a lesser extent, Th17 responses (IL-17 and IL-23) [Cooper et al]. Mtb and other mycobacteria contain well characterized TLR ligands that are potent in vitro stimuli of a number of proinflammatory cytokines including TNF-α and IL-12[Korbel DS et al]. A role for

TLR signaling in host resistance to Mtb is further supported by the observation that mice deficient in MyD88, a major adaptor molecule required for signaling events by most TLR/IL-1R family members, show greatly enhanced susceptibility to aerosol infection with Mtb, equivalent to that observed with IFN-γ-deficient mice [Feng CG et al]. Infected $MyD88^{-/-}$ animals, in addition to their loss of resistance to Mtb, display impaired proinflammatory cytokine synthesis, which was found to correlate with decreased nitric oxide synthase 2 expression and diminished IFN-γ synthesis [Feng CG et al] In addition, TLR2 and 9 have been found to be essential in mediating immunity to Mtb [Bafica A].

Because filarial infections and TB are co-endemic in many parts of the world, we hypothesized that immune responses in latent TB could be modulated by diminished TLR expression and function induced by chronic, coexisting filarial infections. To this end, we examined the baseline and Mtb-specific expression of TLR1, 2, 4, and 9, as well as the induction of pro-inflammatory cytokines by TLR ligands. We observed that the presence of patent filarial infection altered profoundly the TLR-mediated cytokine responses in individuals with coexisting latent Mtb, an immune modulation that is reversible following treatment of the filarial infection.

Study population

We studied a group of 15 patients who were tuberculin skin test positive (PPD^+) but filarial $infection^-$(hereafter PPD^+Fil^-) and 9 who were PPD^+ and filarial $infection^+$ (hereafter PPD^+Fil^+) in Tamil Nadu, South India. Filarial infection was diagnosed by the presence of circulating filarial antigen first by using the ICT filarial antigen test (Binax, Portland, ME, USA) and then confirmed by positivity in the Trop Bio Og4C3 ELISA (Trop Bio Pty. Ltd, Townsville, Queensland, Australia). All

subjects had positive skin test reactivity to intradermal tuberculin (2 TU). A positive tuberculin skin test was defined as an induration at the site of inoculation of at least 12-mm diameter to account for the high prevalence of environmental mycobacteria. This was based on the fact that a rigorous multivariate analysis of 280,000 subjects over a 15-year follow-up had previously demonstrated that subjects with 0–11-mm tuberculin skin test reaction to 2 TU PPD-S comprised the predominantly uninfected group and subjects with 12-mm or greater tuberculin skin test reaction comprised the predominantly infected group in South India. All subjects had normal chest radiographs. None of the subjects had pulmonary symptoms (cough, fever, chest pain, hemoptysis) or a positive sputum for Mtb by smear microscopy and culture. All individuals were examined as part of a clinical protocol (NCT 01-I-N261) approved by Institutional Review Boards of both the National Institute of Allergy and Infectious Diseases and the Tuberculosis Research Center, and informed written consent was obtained from all participants. Fil^+ individuals were treated with diethylcarbamazine (300 mg) per day for 7 days and a single dose of albendazole (400 mg) administered on the first day. Post treatment assessment of immune responses was performed 1 year following treatment.

Characteristics of Study Population

	PPD+Fil- (n=15)	**PPD+Fil+ (n=15)**
Median age (range)	48 (30-66)	42 (25-55)
Gender M/F	7/8	6/9
Treatment	NO	Yes
Tuberculosis Skin Test (TU)	None	None
ICT Card Test	Negative	Positive
W. bancrofti circulating antigen levels U/ml (median)	<32 (<32)	177–32768 (3320)

In vitro culture

PBMCs were cultured with PPD (10 μg/ml) or Mtb CFP (10 μg/ml) or TT (10 μg/ml) in 24-well tissue culture plates (Corning, Corning, NY, USA) at concentrations of 5×10^6/well. After 24 hours, RNA was isolated and examined for TLR gene expression. PBMCs were also cultured with Pam3Cys (10 μg/ml), ultrapure LPS (10 μg/ml), or ODN (5 μM). After 24 hours, culture supernatants were collected and analyzed for cytokines.

ELISA

The levels of cytokines in the culture supernatants were measured using Bioplex multiplex cytokine assay system (Bio-Rad, Hercules, CA, USA). The cytokines analyzed were IFN-γ, TNF-α, IL-12p70, IL-6, and IL-1β.

RNA preparation

PBMCs were lysed using the reagents of a commercial kit (QIAshredder; Qiagen, Valencia, CA, USA). Total RNA was extracted according to the manufacturer's protocol (RNeasy mini kit; Qiagen), and RNA was dissolved in 50 μl of RNase-free water.

cDNA synthesis

RNA (1 μg) was used to generate cDNA using TaqMan reverse transcription reagents according to the manufacturer's protocol (Applied Biosystems, Inc., Fullerton, CA, USA). Briefly, random hexamers were used to prime RNA samples for reverse transcription using MultiScribe reverse transcriptase.

Real-time RT-PCR

Real-time quantitative RT-PCR was performed in an ABI 7500 sequence detection system (Applied Biosystems) using TaqMan Assays-on-Demand reagents for TLR1, 2, 4, and 9 and an endogenous 18 s ribosomal RNA control. Relative transcripts were determined by the formula $½^{(CT\ target-CT\ control)}$

Where CT is the threshold cycle during the exponential phase of amplification.

Statistical analysis

Geometric mean was used as the measure of central tendency. Comparisons were made using either the Mann-Whitney U test (for unpaired data), the Wilcoxon signed rank test (for paired data), or Spearman rank correlation. All statistics were performed using GraphPad Prism version.

Filarial infection is associated with decreased baseline and Mtb-specific TLR2 and 9 expression in latent TB

To determine the impact of coexisting filarial infection on baseline and antigen-specific TLR expression of PPD^+ individuals, and because filarial infection is associated with diminished expression of TLR1, 2, 4, and 9 [Babu S et al], we examined the mRNA expression of these TLRs from PPD^+Fil^- or PPD^+Fil^+ individuals ex vivo as well as following PBMC stimulation with PPD or culture-filtrate antigen from Mtb H37 Rv (Mtb CFP) or TT for 24 hours by RT-PCR. Both baseline as well as PPD- and Mtb CFP-specific induction of TLR2 and 9 was significantly lower in PPD^+Fil^+ patients (TLR2: geometric mean [GM] of 0.7373 in PPD^+Fil^+ vs. 0.7973 in PPD^+Fil^- at baseline [P=0.0340]; GM fold change of 0.9469 in PPD^+Fil^+ vs. 2.513 in PPD^+Fil^- for PPD [P=0.0040]; GM fold change of 0.5866 vs. 4.127 for CFP [P<0.0001] TLR9: GM of 0.5523 vs. 05889 at baseline [P=0.0244]; GM fold change of

0.7660 vs. 2.834 for PPD [P=0.0040]; GM fold change of 0.6989 vs. 2.297 for CFP [P=0.0134]) compared with the $PPD^{+}Fil^{-}$ group. TT-specific TLR expression was examined as a control to TB antigens and did not exhibit any significant difference in expression

Filarial infection is associated with diminished Pam3Cys and ODN - specific pro-inflammatory cytokine production in latent TB

To determine the impact of coexisting filarial infection on TLR-specific pro-inflammatory cytokine responses in PPD^{+} individuals, we stimulated PBMC from $PPD^{+}Fil^{-}$ or $PPD^{+}Fil^{+}$ with Pam3Cys (TLR2 ligand), ODN (TLR9 ligand), or LPS (TLR4 ligand) for 24 hours and measured the levels of IL-1β, TNF-α, IL-6, IL-12, and IFN-γ.Pam3Cys and ODN induced significantly lower levels of IL-1β (GM of 33.60 pg/ml in $PPD^{+}Fil^{+}$ vs. 283.9 pg/ml in $PPD^{+}Fil^{-}$ for Pam3Cys [P=0.0566]; GM of 16.61 pg/ml vs. 9.66 pg/ml for ODN [P=0.0078]), TNF-α (GM of 91.19 pg/ml vs. 338.9 pg/ml for Pam3Cys [P=0.0244]; GM of 76.99 pg/ml vs. 260.1 pg/ml for ODN [P=0.0142]), IL-6 (GM of 511.1 pg/ml vs. 2157 pg/ml for Pam3Cys [P=0.0142]; GM of 90.20 pg/ml vs. 295.6 pg/ml for ODN [P=0.0770, not significant]), IL-12 (GM of 15.17 pg/ml vs. 21.37 pg/ml for Pam3Cys [P=0.0315]; GM of 13.15 pg/ml vs. 32.12 pg/ml for ODN [P=0.0188]) and IFN-γ (GM of 72.54 pg/ml vs. 237.1 pg/ml for Pam3Cys [P=0.0188]; GM of 29.86 pg/ml vs. 294.4 pg/ml for ODN [P=0.0040]) in the $PPD^{+}Fil^{+}$ compared with the $PPD^{+}Fil^{-}$ group. There were no significant differences in the production of the above-mentioned cytokines in response to LPS.

Treatment of filarial infection is associated with enhanced Pam3Cys and ODN - specific pro-inflammatory cytokine production in latent TB

To determine whether anti filarial treatment could reverse the attenuated TLR-specific pro-inflammatory cytokine responses in PPD^+ individuals with concomitant lymphatic filariasis, we stimulated PBMCs from PPD^+Fil^+, pre- and 1 year post-treatment, with Pam3Cys, ODN, or LPS for 24 hours and measured the levels of IL-1β, TNF-α, IL-6, IL-12, and IFN-γ. (and in comparison to pretreatment responses), Pam3Cys and ODN induced significantly increased production of IL-1β (GM of 31 pg/ml in pretreatment vs. 198.3 pg/ml post treatment for Pam3Cys [P=0.0117]; GM of 16.61 pg/ml vs. 47.83 pg/ml for ODN [P=0.0547, not significant]), TNF-α (GM of 91.19 pg/ml vs. 728.2 pg/ml for Pam3Cys [P=0.0273]; GM of 72.73 pg/ml vs. 247.4 pg/ml for ODN [P=0.0195]), IL-6 (GM of 511.1 pg/ml vs. 1396 pg/ml for Pam3Cys [P=0.1289, not significant]; GM of 90.20 pg/ml vs. 418.1 pg/ml for ODN [P=0.0195]), IL-12 (GM of 15.17 pg/ml vs. 105.4 pg/ml for Pam3Cys [P=0.0391]; GM of 13.15 pg/ml vs. 53.46 pg/ml for ODN [P=0.0390]) and IFN-γ (GM of 72.54 pg/ml vs. 201.4 pg/ml for Pam3Cys [P=0.0195]; GM of 27.89 pg/ml vs. 174.6 pg/ml for ODN [P=0.0273]) in the PPD^+Fil^+ group. Filarial infection treatment had minimal impact on LPS-induced production of the above-mentioned cytokines except IL-1β (P=0.0391), which also showed an increase following antifilarial treatment. Finally, there was a strong relationship between the decrease in the circulating filarial antigen levels, a surrogate marker of treatment efficacy, and the increase in TNF-α and IFN-γ levels in response to Pam3Cys (P=0.0045 and r=0.8667 for TNF-α, and P=0.0311 and r=0.7333 for IFN-γ) and to ODN (P=0.0061 and r=0.8500 for TNF-α, and P=0.0045 and r=0.8667 for IFN-γ) following ant filarial treatment.

Filarial infection is associated with diminished cytokine responses to TLR2 and TLR9 ligands in latent TB patients.

Stimulations	$PPD^{+}Fil^{-}$
Baseline	TLR-2 ↑ TLR-9 ↑
PPD	TLR-2 ↑ TLR-9 ↑
CFP	TLR-2 ↑ TLR-9 ↑

Filarial infection is associated with diminished cytokine responses to TLR2 and TLR9 ligands in latent TB patients.

Stimulations	CP
Pam3Cys	TNF-a ↑ IFN-g ↑ IL-1b ↑ IL-6 ↑ IL-12 ↑
ODN	TNF-a ↑ IFN-g ↑ IL-1b ↑ IL-6 ↑ IL-12 ↑

Discussion

Parasitic helminths have evolved mechanisms to overcome and evade host immune responses to thrive in immune-exposed locations such as lymphatics, bloodstream, and gastrointestinal tract [Maizels et al]. Most helminth infections induce relatively little disease in spite of extraordinarily high loads of infection. This subversion of the host immune response is achieved through induction of multiple layers of immuno-regulation [Maizels et al.] The interactions of helminth parasites with APCs are known to involve TLRs [Venugopal et al], pattern-recognition receptors that form a key component of microbial detection and host defense and are important in the

initiation of host immune responses [Takeda et al]. TLRs are involved in recognition of a wide spectrum of pathogens by binding to pathogen-associated molecular patterns [Barton et al]. In addition, TLRs control multiple APC functions and activate signals critically involved in the initiation of adaptive immune responses [Iwaski et al]. Downregulation of TLR-mediated immune responses—through dampening TLR-mediated cell signaling or through diminished TLR expression—appears to be an important immune evasion mechanism in some bacterial pathogens as well as in helminth infections [Venugopal PG et al]. Thus, children with schistosomiasis have diminished responses to TLR ligands compared with uninfected children in the same endemic area [Vander Kleijii et al]. Similarly, individuals with filarial infection have diminished expression of APC- and T cell-specific-TLR1, 2, 4, and 9 as well as decreased pro-inflammatory cytokine responses to TLR2, 4, and 9 ligands [Babu S et al].

Because immune-mediated protection against Mtb is characterized by strong Mycobacterium-specific Th1 responses [Salgame et al], it has been postulated that coincident infections with helminth parasites could modulate these immune responses by driving Th2 and/or regulatory T cells that induce anti-inflammatory responses [Van Riet et al] . Indeed, we have previously shown that filarial infection coincident with Mtb significantly diminishes Mtb-specific Th1 (IL-12/IFN-γ) and Th17 (IL-23/IL-17) responses related to increased expression of CTLA-4 and PD-1 [Babu S et al]. Others have also shown that the poor immunogenicity of bacillus Calmette-Guérin vaccination in helminth-infected populations is associated with elevated TGFβ production [Elias et al]. Because filarial infections and Mtb infections are highly co-endemic in many parts of the world and often coexist within the same host, we wanted to examine the effect of filaria-induced downmodulation of TLRs on host responses to Mtb. In addition, the

systemically circulating microfilariae may sequester in lung capillaries when not in the bloodstream, allowing them to be localized to the anatomic compartment associated with Mtb infection. Activation of the innate immune system via a number of pattern-recognition receptors, including TLRs, is thought to be a prerequisite for driving a protective adaptive immune response to Mtb [Korbel DS et al]. Several studies have shown that Mtb contains a variety of pathogen-associated molecular patterns that serve as ligands for TLRs [Korbel DS et al]. Based on a variety of in vitro studies, it has been suggested that TLR2, 4, and 9 are critically involved in induction of a Th1 response following infection with Mtb [Korbel DS et al]. However, in vivo data are not as clear cut. While some studies have shown no role for individual TLRs in protection against Mtb [Feng et al]others have shown that TLR2 and 9 are crucial in host resistance to Mtb [Bafica et al].

Our study reveals a new mechanism by which coexisting filarial infections can modulate immune responses to Mtb infections. Dually infected individuals exhibit a significant decrease in the baseline as well as Mtb antigen-induced expression of TLR2 and 9. While the baseline decrease in expression of TLR2 and 9 mRNA has been shown by us previously, the impact on antigen-driven TLR expression is striking. The diminished ability to upregulate TLR expression following exposure to Mtb antigens suggests that individuals with filarial infections would be impaired in their immune response to TB and be at significant risk to develop active disease. The examination of cytokine responses to Toll-ligands in $PPD^{+}Fil^{+}$ patients revealed interesting differences in comparison with $PPD^{+}Fil^{-}$ individuals. First, IFN-γ and IL-12 were significantly downregulated in $PPD^{+}Fil^{+}$ individuals, suggesting that the IL-12/INF-γ pathway in patients with coincident lymphatic filariasis and latent TB is compromised. This has important clinical relevance, in that it is well known that mutations in the IL-12-IFN-γ–

Stat1 pathway can lead to disseminated TB and atypical mycobacterial infections in humans [Dorman SE et al]; in addition, mice deficient in IL-12 and/or IFN-γ are more susceptible to Mtb infection than their WT controls [Chan J et al]. IFN-γ is the central effector molecule in macrophage elimination of bacteria, in that it induces increases in reactive nitrogen and oxygen compounds responsible for bactericidal activity as well as being central in the induction of autophagy, a process recently documented to play a critical role in eliminating mycobacteria within dendritic cells and macrophages [Chan J et al]. Second, TNF-α production is significantly impaired in $PPD^{+}Fil^{+}$ individuals compared to the $PPD^{+}Fil^{-}$individuals. TNF-α is another cytokine that plays an important role in preventing development of active clinical disease in individuals with latent TB [Stenger et al]. Treatment of autoimmune diseases with TNF-α antagonists results in reactivation of Mtb and development of clinical disease in these individuals [Ehlers et al]. Thus, compromised production of TNF-α in response to Toll ligands suggests another mechanism that predisposes individuals with filarial infection to develop active TB. Finally, $PPD^{+}Fil^{+}$ individuals also exhibit significantly decreased production of IL-1β and IL-6 in comparison to $PPD^{+}Fil^{-}$ individuals in response to TLR ligands. Because IL-1β and IL-6 are important proinflammatory cytokines necessary for recruitment of innate effector cells such as macrophages, polymorphonuclear neutrophils, and NK cells to the infectious foci, the lack of induction of these cytokines would result in compromised activation of an effective adaptive immune response to Mtb [Korbel DS et al].. In addition, IL-1β has also been shown to be essential for protection against Mtb infection in mice [Fremond et al]. Thus, diminished functional responses to TLR2 and 9 ligands could potentially disrupt multiple mechanistic pathways operational in the control of Mtb infection.

Interestingly, treatment of filarial infection with a regimen of DEC and albendazole, resulted in significant lowering of worm burdens at 1 year post treatment as evidenced by the decrease in circulating filarial antigen levels. This is accompanied by the restoration of TLR-mediated cytokine responses to Pam3Cys and ODN. Thus, the diminished pro-inflammatory cytokine production observed in $PPD^{+}Fil^{+}$ individuals is abrogated following treatment, suggesting that the associated filarial infections are the cause of lowered cytokine responses in latent TB individuals. Moreover, there is a direct correlation between the decrease in filarial antigen load following treatment and the quantitative restoration of the cytokine responses in these individuals. Our findings highlight a novel mechanism by which a systemic helminth infection can modulate the cytokine response to Mtb through its effects on TLRs.

Thus, alteration of TLR expression and function in filaria-infected individuals with latent TB can have major implications in the control of latent TB infection. In addition, these findings also have significant implications for vaccine efficacy in helminth-endemic countries. Vaccines requiring a pro-inflammatory cytokine response for efficacy and those involving TLR agonists as adjuvants may not function optimally in the presence of helminth coinfection. The reversal of TLR modulation upon treatment of filarial infection suggests that elimination of this helminth infection in endemic areas might have a profound effect in the control of TB infection.

Co-infection studies

This study was carried out in three panchayats consisting a population of more than 5,000 in PoondiPanchayat Union of TiruvelloreTaluk with an intense geohelminth infections and active filarial infection of 10-25%. The sample size was estimated about 5200 consisting of both males and females, in the age group 6 to 65 year old group.

All individuals were registered as per the epidemiology unit work procedures and the information will be recorded on individual card. Individuals aged 6-65 years were eligible for blood collection as well as stool examination. Disease status and information on passing of worms was collected by census taker during registration. If the individual was absent during census, this information was collected at the centre, where all eligible persons are directed for blood collection using ICT Diagnostic Kit as per the instruction given in the kit. Group and individual number were recorded on the cover of the kit at the time of blood collection with sketch pen. After the blood collection at the centre, a container was issued to each asking them for a sample of stool on the same day or overnight. The group and individual number were recorded on the cap as well as on the sticker pasted on the container using magic marker before issuing the container to the individual for stool collection. The overnight specimen was collected on the next day by the morning team.

On an average, 80-100 blood and stool specimens were collected in a day.

Results:

Results for co-infection studies are given in Annexure-1

Summary and Conclusion

SUMMARY AND CONCLUSIONS

- Lymphatic filariasis is a major health problem in many countries of the world. The disease still takes a heavy toll in terms of social and economic losses.

- The commonest clinical forms seen in a large clinic in an endemic area are lymphoedema of the lower limbs, hydrocoele, lymphoedema of the genitalia, chyluria and tropical eosinophilia.

- Apart from filarial oedema, the most important clinical manifestation of filariasis is the episodes of adenolymphangitis. The brief duration and episodic nature of ADL makes it the best manifestation of lymphatic filarial disease that can be well studied.

- These ADL episodes contribute significantly to the morbidity associated with the disease. Recurrent attacks of lymphangitis lead on to the progression of lymphoedema and development of elephantiasis

- Patients with higher grades of lymphoedema were more predisposed to ADL attacks. Thus, while there were ADL episodes in patients with Grade III oedema, they were only in grade II and in Grade I. This can be explained by the predisposition to fungal infection in limbs with higher grades of oedema.

- This study has shown that any factor resulting in injury to the skin of the oedematous limb promotes secondary bacterial infection, which is the most important cause for the ADL episodes. Fungal infection of the affected limb, especially on constant exposure to moisture, is the commonest predisposing factor, followed by minor injuries and infections.

- As in the case of the analysis done according to drug groups there was an increase in the number of ADL attacks in the follow up phase when compared to the treatment year. A response in the form of reduction in the episodes of ADL in the treatment groups; oral penicillin group alone, DEC group alone and the combination; as well as the foot care group was significant ($p<0.008$). This was however statistically significant only for patient with grade III ($p<0.025$) and grade IV ($p<0.009$) oedema.

- The reductions were greater in the lower grades of oedema as compared with the higher grades although the difference between the groups were not statistically significant. There was an increase in the number of attacks in the follow up year.

- The importance of the role of bacteria entering through breaks in the skin and precipitating ADL attacks has been convincingly demonstrated.

- Further long-termstudies, which would help to document that the lymphoedema of early filarial oedema can be reversed by using antibiotics and carefully designed limb care programmes. This beneficial effect would help to enhance the success of large-scale control programmes since the perceived benefit by the affected persons would be high.

- Advanced Multi modality treatment for the reversal of grade IV lemphedema patient has been performed with nodovenal shunt in order to get best outcome in cosmetic and functional limb.

- Helminth infections in the study area were highly prevalent. Geohelminths and filarial infections were seen maximally in the younger years of life.

- PPD sensitivity was influenced by the presence of helminth infections.

- Clearance of helminth infections may provide better response to a variety of vaccines.

- Targeting of these pathways, as well as new treatment regimens involving TLR modulation, could potentially provide new avenues in ameliorating pathology in chronic lymphatic filariasis. In addition, our data also reveal a new link between innate immune responses (in the form of TLR activation) and angiogenic/ lymphangiogenic pathways (in the form of elevated growth factors), thereby providing additional insight into the pathways connecting the immune system and normal physiologic processes such as lymphatic circulation.

- Alteration of TLR expression and function in filaria- infected individuals with latent TB can have major implications in the control of latent TB infection. In addition, these findings also have significant implications for vaccine efficacy in helminth- endemic countries. Vaccines requiring a pro-inflammatory cytokine response for efficacy and those involving TLR agonists as adjuvants may not function optimally in the presence of helminth coinfection. The reversal of TLR modulation upon treatment of filarial infection suggests that elimination of this helminth infection in endemic areas might have a profound effect in the control of TB infection.

Significance of the Study

SIGNIFICANCE OF THE STUDY

Lymphatic filariasis (LF) is a chronically disabling and debilitating mosquito-borne parasitic infection that infects an estimated 120 million persons worldwide. LF is endemic in 81 countries with approximately more than 1.3 billion people at risk of infection worldwide. The Global Programme for the Elimination of Lymphatic Filariasis (GPELF) was launched in 2000, and as of 2015 scaled up to introduce MDA in 53 endemic countries. The GPELF focuses on the interruption of transmission through mass drug administration and managing and preventing disability, such as ADL, lymphedema, and hydrocele, for infected individuals.

Since the inception of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 2000, most of the attention on disease elimination has focused on the interruption of disease transmission with efforts targeting morbidity taking second stage. However, with mass drug administration (MDA) programs successfully reducing microfilaremia rates in targeted communities, more attention has been placed on the need to follow the long term disabling effects of filarial disease, namely adenolymphangitis (ADL), hydrocele and lymphedema. This awareness has led to an increased number of studies incorporating morbidity assessment as an outcome for monitoring the success of MDA. Additionally, GPELF has increased its efforts regarding response to morbidity related issues. Activities based on morbidity prevention have involved educational events at local hospitals, community wide trainings, and patient care tutorials within the context of complex and diverse settings. Most of these actions have occurred as the result of previous research focusing on the benefits of interventions like basic hygiene on preventing physical disease progression. Despite this increase in awareness, morbidity improvement, in particular, has not been considered in depth as a consequence of MDA, and existing literature indicates there is

little consistency on how to regard previous research and the best way to approach future assessments in a standardized manner.

- ✓ Lymphatic filariasis is a neglected mosquito-borne tropical disease which caused by filarial worms, *Wuchereria bancrofti*, *Brugia malayi* or *B. timori* It is endemic in 58 countries, putting 1.2 billion people at risk globally with an estimated 120 million infected
- ✓ Filariasis causes relatively low mortality but has a high incidence of morbidity that has a major social impact, causing heavy economic loss in developing countries
- ✓ It is the second leading cause of permanent or long-term disability with over 40 million infected people suffering from pathological manifestations like lymphoedema, hydrocoele, chyluria and elephantiasis
- ✓ The standard method for diagnosing active infection is by finding the microfilariae via microscopic examination. This may be difficult, as in most parts of the world; microfilariae only circulate in the blood at night. For this reason, the blood has to be collected nocturnally. The blood should be in the form of a thick smear and stained with Giemsa. Testing the blood for filarial antigen has been transformative in enabling the rapid and accurate diagnosis of LF.
- ✓ The clinical manifestations of LF are varied, Traditionally, it has been accepted that people living in an endemic area can be classified into five groups: (1) Endemic Normals; (2) clinically asymptomatic, infected; (3) Acute clinical disease; (4) Chronic pathology and (5) Tropical pulmonary eosinophilia (TPE)
- ✓ While filarial infection does induce expression of immune cells in humans, early interaction of parasites or parasite antigens leads to a predominantly pro-

inflammatory response with expression of mainly pro-inflammatory cytokines including TNFα, IL-6 and IL-1β, as well as genes involved in inflammation and adhesion

- ✓ Increase in circulating levels of MMPs and TIMPs is characteristic of the filarial disease process and that that altered ratios of MMP/TIMP are an important underlying factor in the pathogenesis of tissue fibrosis in filarial lymphatic disease
- ✓ A basic, recommended package of care can alleviate suffering and prevent further disability among lymphatic filariasis patients.
- ✓ Advanced Multi modality treatment for the reversal of grade IV lemphedema patient can be recommended to get best outcome in cosmetic and functional limb.
- ✓ Targeting of these pathways, as well as new treatment regimens involving TLR modulation, could potentially provide new avenues in ameliorating pathology in chronic lymphatic filariasis. In addition, our data also reveal a new link between innate immune responses (in the form of TLR activation) and angiogenic/ lymphangiogenic pathways (in the form of elevated growth factors), thereby providing additional insight into the pathways connecting the immune system and normal physiologic processes such as lymphatic circulation.
- ✓ Alteration of TLR expression and function in filaria-infected individuals with latent TB can have major implications in the control of latent TB infection. In addition, these findings also have significant implications for vaccine efficacy in helminth- endemic countries.
- ✓ Vaccines requiring a pro-inflammatory cytokine response for efficacy and those involving TLR agonists as adjuvants may not function optimally in the presence

of helminth coinfection.

- ✓ The reversal of TLR modulation upon treatment of filarial infection suggests that elimination of this helminth infection in endemic areas might have a profound effect in the control of TB infection.
- ✓ Treatments for lymphatic filariasis differ depending on the geographic location of the endemic area. Whereas elsewhere in the world, albendazole is used with diethylcarbamazine.
- ✓ Geotargeting treatments is part of a larger strategy to eventually eliminate lymphatic filariasis by 2020

Lymphatic filariasis can be eliminated by stopping the spread of infection through preventive chemotherapy with single doses of 2 medicines for persons living in areas where the infection is at present

Future Scope

FUTURE SCOPE IN THE FIELD OF LYMPHATIC FILARIASIS AND CO-INFECTIONS

TB - HELMINTHIASIS – VACCINE RESEARCH

Doxycycline again provides an alternate therapy against *W. bancrofti* and *B. malayi* (Supali T et al 2009**,**Hoerauf A et al 2011) with the additional benefit of its ability to ameliorate pathology (Debrah AY et al 2006**,**Mand S et al 2009). However, doxycycline cannot be given to children under the age of 9 (Hoerauf A et al 2008) who, together with pregnant women and lactating mothers, are excluded from mass treatment programmes. Children below 5 years are also excluded from ivermectin treatment. This leaves a significant proportion of individuals exposed to infection. For example, in Gabon where the entire country is co-endemic for *O. volvulus* and *L. loa* (Zoure HG et al 2010), 20% of the population is under 10 years (United Nations, http://esa.un.org/unpd/wpp/index.htm), and similar age profiles are found throughout filarial endemic regions of Africa. Therefore, untreated children represent a large reservoir of microfilariae that can contribute to transmission. Furthermore, for the individual, the consequences of not receiving treatment would be the prospect of developing progressive filarial disease and more general long-term health problems as well as associated socio-economic disadvantage.

Despite these obstacles, there is a room for optimism. The existence of natural immunity in people gives hope that vaccines can be developed as does the success of vaccines in animal models. Knowledge of antifilarial immunity has made enormous advances in recent years, and development of the rodent filarial nematode *L. sigmodontis* has allowed the full power of mouse genetics and immunology to be applied to antifilarial vaccine research. At the same time, the introduction of high-

throughput technologies enables examination of the entire molecular repertoires of both parasite and hosts. Combined with the application of system analyses, these data are being used to identify the pathways that induce and regulate protective immunity. Furthermore, this combination also identifies traits that can lead to pathology, and the evolutionary and ecological forces driving potential vaccine failures. Our best hopes may lie with designing vaccines that do not exist naturally, i.e. genetically modified antigens, and adjuvants that decouple immunopathology from protective immune responses.

Over 2 billion people worldwide are infected with helminths (worms). Similarly, infection with *Mycobacterium tuberculosis* (Mtb) occurs in over a third of the world's population, often with a great degree of geographical overlap with helminth infection. Interestingly, the responses induced by the extracellular helminths and those induced by the intracellular Mtb are often mutually antagonistic and, as a consequence, can result in impaired (or cross-regulated) host responses to either of the infecting pathogens. In this review, we outline the nature of the immune responses induced by infections with helminths and tuberculosis (TB) and then provide data from both experimental models and human studies that illustrate how the immune response engendered by helminth parasites modulates Mtb-specific responses in helminth–TB coinfection.

Helminths have evolved complex mechanisms for immune subversion, with effects on both adaptive and innate immune responses that lead to their long-term persistence. Spillover effects on mycobacterial antigen responses have been seen in both in vitro and in vivo studies. No clear consensus has, however, emerged on whether this affects vaccine responses and enhances susceptibility to active TB disease.

Although animal models provide important insights into specific pathways of immunomodulation, human studies have suffered from multiple constraints. Prospective studies following large cohorts with serial assessment for both helminth infection status as well as for development of active TB disease are logistically challenging to perform. Future studies will therefore need stringent definitions for inclusion criteria that incorporate the use of highly sensitive and specific diagnostic tools and clear enumeration of confounding variables like HIV, polyparasitism, and measures of intensity and stage of infection.

There is a substantial portfolio of interesting TB vaccine candidates in clinical phase-I/II testing, some of which are already fairly advanced in the TB vaccine pipeline. Numerous promising candidates further upstream in the pipeline complement this. In fact, an emerging bottleneck may not be the number of pre-clinical TB vaccine candidates that TB researchers can produce, but rather the number of vaccines that can be tested clinically in efficacy trials, given the limited clinical trial capacity worldwide, i.e., a shortage that exists not only in Africa but also in Asia. Finally, the identification of TB surrogate end-point biomarkers or “correlates of protection" may drastically reduce the need for the current long-term large-scale clinical trials, and thus will speed up TB vaccine discovery and clinical testing.

References

REFERENCES

A Krishna Kumari, KT Harichandrakumar, KL Das, K Krishnamoorthy. Physical and psychosocial burden due to lymphatic fialriasis as perceived by patients and medical experts. *Trop Med Int Health* 2005; 10: 567-73.

AC Dhariwal, PK Srivastava, J Bhattacharjee Journal of the Indian Medical Association, Vol. 113, No. 12, December 2015)

Activity. *London: Wellcome Trust Publishing* 1st edition. 1996.

Addiss D, Fiack C, Lammie PJ, Alexis CT, Saint Louis G, Radday J,McLaughlin SI: Occurrence of painful scrotal nodules and hydrocele following mass treatment with diethylcarbamazineand albendazole for lymphatic filariasis. *Am J Trop Med Hyg* 2001, 63(Supplement 3, Abstract 792):424

Adhikari P, Haldar JP. Prevalence of bancroftian filariasis in Burdwan district. West Bengal: II. Vector and microfilariae density in colliery and non-colliery areas. J Commun Dis 1995; 27(3): 181-185.

Adhikari P, Haldar S, Ghosh NR, Mandal MM, Haldar JP. Prevalence of Bancroftian filariasis in Burdwan District, west Bengal: a comparative study between colliery nd non-colliery areas. J Commun Dis 1994; 26(1):6-13.

Adolescent girls in rural Nigeria. WHO/TDR Discussion Paper. Geneva:

Agarwal M, Prasad GB, Harinath BC, Bhatia BD. Transplacental transfer of filarial infection. Indian Pediatr 1986; 23(3): 169-174.

Agarwal SK, Mitra MK, Misra R, Sethi PP, Murthy PK, Chatterjee RK. Comparative evaluation of immunodiagnostic tests in lymphatic filariasis using Brugia malayi adult whole worm antigen. J Assoc Physicians India 1987; 35(7):507-509.

Agrawal MC, Shah HL. Stephanofilarial dermatitis in India. Vet Res Commun 1984; 8(2):93-102.

Ahamed B, Sivakumar MR, Alamelu R, Kumarswami Y, Naravanan PR, Thiruvengadam KV. Possible sensitization by filarial antigens in diethylcarbamazine (DEC) responsive bronchial asthma. A preliminary enquiry. J Assoc Physicians India 1984; 32(ll):937-938.

Ahorlu CK, Dunyo SK, Simonsen PE. Scarification as a risk factor for rapid

Alexander N, Perry R, Dimber Z, Hyun P, Alpers M, Kazura J: Acute disease episodes in a *Wuchereria bancrofti*-endemic area of Papua New Guinea. Am J Trop Med Hyg. 1999, 61 (2): 319-324.

Alexopoulou, L., A. C. Holt, R. Medzhitov, and R. A. Flavell. 2001. Recog- nition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor Nature 413:732–738.

Allen JE and Maizels RM. Diversity and dialogue in immunity to helminths. *Nat. Rev.Immunol* 2011; 11:375–388.

Al-Shaham AA, Sood S. Recurrent furunculosis as a cause of isolated penile lymphedema: a case report. J Med Case Reports 2010; 4: 196.

Amaral F, Dreyer G, Figueredo-Silva J, Noroes J, Cavalcanti A, Samico SC, Santos A, Coutinho A. Live adult worms detected by ultrasonography in human Bancroftian filariasis. Am J Trop Med Hyg. 1994; 50:753–757. [PubMed: 8024070]

Amaral F, Dreyer G, Figueredo-Silva J. Live adult worms detected by ultrasonography in human bancroftian filariasis. Am J Trop Med Hyg 1994; 50: 735-757.

Amat-Roze JM: Geographic inequalities in HIV infection and AIDS in sub-

Amazigo UO, Obikeze DS. *Social consequences of onchocercal skin lesions on*and chronic forms of lymphatic filariasis in urban areas in south India. *Trop Med Int Health* 2003; 8: 56-9.

Anderson J, Maclean M, Davies C: Malaria research. An audit of international

Anderson J: Filariasis in British Guiana. London School of Tropical Medicine, Research Memoir Series. London School of Medicine. 1924

Anitha, K Shenoy RK. Treatment of lymphatic filariasis. Curr Trends Continuing Medical Education 2001: 67(2): 60-65.

Annual Report, Vector Control Research Centre, Pondicherry. p18, 1998.

Annual Report, Vector Control Research Centre, Pondicherry. p23, 2000.

Anuradha R, George PJ, Chandrasekaran V, Kumaran PP, Nutman TB, Babu S. "Interleukin 1 (IL-1)- and IL-23-mediated expansion of filarial antigen-specific Th17 and Th22 cells in filarial lymphedema." Clin Vaccine Immunol. 2014 Jul;21(7):960-5. doi: 10.1128/CVI.00257-14

Anuradha R, George PJ, Hanna LE, Chandrasekaran V, Kumaran P, Nutman TB, Babu S. "IL-4-, TGF-β-, and IL-1-dependent expansion of parasite antigen-specific Th9 cells is associated with clinical pathology in human lymphatic filariasis." J Immunol. 2013 Sep 1;191(5):2466-73. doi: 10.4049/jimmunol.1300911

Anuradha R, George PJ, Hanna LE, Chandrasekaran V, Kumaran PP, Nutman TB, Babu S. "Parasite-antigen driven expansion of IL-5(-) and IL-5(+) Th2 human subpopulations in lymphatic filariasis and their differential dependence on IL-10 and TGFβ." PLoS Negl Trop Dis. 2014 Jan 30;8(1):e2658. doi: 10.1371/journal.pntd.0002658

Anuradha R, George PJ, Kumaran P, Nutman TB, Babu S. "Interleukin-10- and transforming growth factor β-independent regulation of $CD8^+$ T cells expressing type 1 and type 2 cytokines in human lymphatic filariasis." Clin Vaccine Immunol. 2014 Dec;21(12):1620-7. doi: 10.1128/CVI.00598-14

Anuradha R, George PJ, Kumaran P, Nutman TB, Babu S: Circulating microbial products and acute phase proteins as markers of pathogenesis in lymphatic filarial disease. PLoS Pathog. 2012.

Anuradha R: Altered circulating levels of matrix metallproteinases and inhibitors associated with elevated Type 2 cytokines in lymphatic filarial disease. PLoS Negl Trop Dis. 2012.

Appavoo, N.C., Pani S.P. and Das, P.K. The Science and Art of Lymphatic Lymphology 43:59 –72.

Arora BS, Kumar S, Mathur MD. Filariasis as a cause of pleural effusion. Indian J Pathol Microbiol 2000; 43(4):491-492.

Arunachalam N, Mariappan T, Vijayakumar KN, Sabesan S, Panicker KN. Mattancherry urban agglomeration, a diminishing focus of lymphatic filariasis in Kerala. J Commun Dis 1996; 28(3): 168-170.

Ayres M, Salzano FM, Helena M, Franco LP, Souza Barros RM. The association of blood groups, ABH secretion, haptoglobins and hemoglobins with filariasis. HumHered 1976; 26(2): 105-109.

B Carme, A Utahia, E Tuira, T Teuru. Filarial elephantiasis in French Polynesia: a study concerning the beliefs of 127 patients about the origins of their disease.

B Haliza, R Mohd. Comparison of knowledge on filariasis and epidemiological

B Nanda, K Krishnamoorthy. Treatment seeking behaviour and costs due to acute

Babu BU, Kar SK (2004) Coverage, compliance and some operational issues of

Babu BV, Acharya AS, Mallick G, Jangid PK, Nayak AN, Satyanarayana K. Lymphatic filariasis in Khurda district of Orissa, India: an epidemiological study. Southeast Asian J Trop Med Public Health 2001; 32(2):240-243.

Babu BV, Nayak AN, Dhal K: Epidemiology of episodic adenolymphangitis: A longitudinal prospective surveillance among a rural community endemic for bancroftian filariasis in coastal Orissa, India. BMC Public Health. 2005, 5 (50)

Babu S, Blauvelt CP, Kumaraswami V, Nutman TB. 2006. Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. J. Immu- nol. 176:3248 –3256.

Babu S, Blauvelt CP, Kumaraswami V, Nutman TB. Chemokine receptors of T cells and of B cells in lymphatic filarial infection: a role for CCR9 in pathogenesis. J Infect Dis. 2005; 191:1018– 1026. [PubMed: 15717282]

Babu S, et al. 2009. Filarial lymphedema is characterized by antigen- specific Th1 and Th17 proinflammatory responses and a lack of regulatory T cells. PLoS Negl. Trop. Dis. 3:e420. doi:10.1371/journal.pntd.0000420.

Babu S, Kumaraswami V, Nutman TB. Alternatively Activated and Immunoregulatory Monocytes in Human Filarial Infections. J Infect Dis. 2009; 199:1827–1837. [PubMed: 19456233]

Babu, S., C. P. Blauvelt, V. Kumaraswami, and T. B. Nutman. 2005. Dimin- ished expression and function of TLR in lymphatic filariasis: a novel mech- anism of immune dysregulation. J. Immunol. 175:1170–1176.

Babu, S., C. P. Blauvelt, V. Kumaraswami, and T. B. Nutman. 2006. Cutting edge: diminished T cell TLR expression and function modulates the immune response in human filarial infection. J. Immunol. 176:3885–3889.

Babu, S., C. P. Blauvelt, V. Kumaraswami, and T. B. Nutman. 2006. Regu- latory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. J. Immu- nol. 176:3248–3256.

Babu, S.,et al. 2009. Filarial lymphedema is characterized by antigen-specific Th1 and Th17 proinflammatory responses and a lack of regulatory T cells. PLoS Negl. Trop. Dis. 3:e420.

Bacteriologic studies of skin, tissue fluid, lymph, and lymph nodes in patients with filarial

lymphedema. *Am J Trop Med Hyg* 1997, 57(1):7-15.

Badhe BP, Sane SY. Human pulmonary dirofilariasis in India: a case report. J Trop Med Hyg 1989; 92(6):425-426.

Bagayoko M, Clarke GP, Craig M, Le Sueur D: A spatial statistical approach to malaria mapping. *Int J Epidemiol* 2000, 29:355-61.

Baird J, Charles J, Streit T, Roberts J, Addiss D, Lammie P: Reactivity to bacterial, fungal, and parasite antigens in patients with lymphedema and elephantiasis. Am J Trop Med Hyg. 2002, 66 (2): 163-169.

Bal M, Das MK. Antibody response to a filarial antigen fraction in individuals exposed to Wuchereria bancrofti infection in India. Acta Trop 1999; 72(3):259-274.

Bal M, Das MK. Antigenicity of a filarial protease from Setaria digitata in Wuchereria bancrofti infection. Ann Trop Med Parasitol 1999; 93(3):279-288.

Bal M, Satapathy AK, Das MK. Increased antibody response to parasite lipids in amicrofilaraemic individuals from a region where Wuchereria bancrofti infection is endemic. Ann Trop Med Parasitol 1998; 92(1):119-122.

Balakrishnan N, Ramaiah KD, Pani SP. Efficacy of bi-annual administration of DEC in the control of bancroftian filariasis. J Commun Dis 1992; 24(2):87-91.

Bancroftian filariasis prevalence by family size in East Godavari district (Andhra Pradesh). J Commun Dis 1981; 13(4):281-283.

Bandyopadhyay L. Lymphatic filariasis and the women of India. Soc Sci Med 1996; 42(10):1401-1410.

Baruah K, Rai RN. The impact of housing structures on filarial infection. Jpn J Infect Dis 2000; 53(3): 107-110.

Basu S, Wanchoo M, Aggarwal A, Malhotra S. Filaria associated hemolysis in pregnancy. Int J Gynaecol Obstet 2000; 69(l):51-52.

Beaver PC, Fallon M, Smith GH. Pulmonary nodule caused by a living Brugia malayi-like filaria in an artery. Am J Trop Med Hyg 1971; 20(5):661-666.

Beg MA, Naqvi A, Zaman V, Hussain R. Tropical pulmonary eosinophilia and filariasis in Pakistan. Southeast Asian J Trop Med Public Health 2001; 32(1):73-75.

Beg MA. Risk of imported filariasis in Pakistan. J Pak Med Assoc 2000; 50(l):33-35.

Bennuru S, Maldarelli G, Kumaraswami V, Klion AD, Nutman TB. Elevated levels of plasma angiogenic factors are associated with human lymphatic filarial infections. Am. J. Trop. Med. Hyg. 83:– 890.

Bennuru S, Maldarelli G, Kumaraswami V, Klion AD, Nutman TB: Elevated levels of plasma angiogenic factors are associated with human lymphatic filarial infections. Am JTrop Med Hyg. Oct 2010;83:884–890.

Bennuru S, Nutman TB. 2009. Lymphangiogenesis and lymphatic re- modeling induced by filarial parasites: implications for pathogenesis. PLoS Pathog. 5:e1000688. doi:10.1371/journal.ppat.1000688.

Bennuru S, Nutman TB: Lymphangiogenesis and lymphatic remodeling induced by filarial parasites: implications for pathogenesis. PLoS Pathog. Dec 2009;5:e1000688.

BennuruS,NutmanTB.2009.Lymphatics in human lymphatic filariasis: in vitro models of parasite-induced lymphatic remodeling. Lymphat. Res. Biol. 7:215–219.

Bera DK, Banerjee P. A lymphatic filarial survey revealing a focus of Brugia malayi in the coastal region of west Bengal. J Indian Med Assoc 1996; 94(8) :296-7,310.

Beuria MK, Bal M, Das MK. Allergic reactivity and IgG subclasses to a proteinase fraction of Setaria digitata in filariasis. J Helminthol 1995; 69(3): 181-185.

Bhattacharjee ML, Dass DD. The study of ear sore of buffaloes due to stephanofilariasis in Assam. Indian Vet J 1967; 44(5):400-402.

Bhattacharya C, Singh RN, Misra S, Rathaur S. Diethylcarbamazine: effect on lysosomal enzymes and acetylcholine in Wuchereria bancrofti infection. Trop Med Int Health 1997; 2(7):686-690.

Bhattacharya NC, Gubler DJ. A survey for bancroftian filariasis in the Calcutta area. Indian J Med Res 1973; 61(1):8-11.

Bhattacharya P. Regional epizootiology and prophylaxis of parasitic diseases (India). BuU Off Int Epizoot 1968; 69(1): 191-193.

Bilharziasis Laboratory, Denmark & National Institute for Medical Research; 1997.

Biswas H, Sharma SP, Das M, Rao VG, Yadava RL, Narasimham MV. Filariasis control in rural areas through detection and treatment with diethylcarbamazine. J Commun Dis 1989; 21(4):272-281.

Biswas SK, Saha SC, Choudhury M. Letter: A new species of microfilaria? Lancet 1975; 1(7901):285.

Bonnetblanc J, Bedane C: Erysipelas – recognition and management. *Am J Clin Dermatol* 2003, 4:157-163.

Borst GM, Goettler CE, Kachare SD, Sherman RA. Maggot Therapy for Elephantiasis Nostras Verrucosa Reveals New Applications and New Complications: A Case Report. *Int J Low Extrem Wounds*. 2014 May 25. 13(2):135-139

Boyd J, Sloan S, Meffert J. Elephantiasis nostrum verrucosa of the abdomen: clinical results with tazarotene. *J Drugs Dermatol*. 2004 Jul-Aug. 3(4):446-8. .

Brattig NW, et al. 2004. The major surface protein of Wolbachia endo- symbionts in filarial nematodes elicits immune responses through TLR2 and TLR4. J. Immunol. 173:437– 445.

Brenchley JM, Douek DC: Microbial translocation across the GI tract. Annu Rev Immunol. 2012;30:149 173.

Bundy DA, Grenfell BT, Rajagopalan PK. Immunoepidemiology of lymphatic filariasis: the relationship between infection and disease. Immunol Today 1991; 12(3):A71-A75.

Burri H, Loutan L, Kumaraswami V, Vijayasekaran V. Skin changes in chronic lymphatic filariasis. Trans R Soc Trop Med Hyg 1996; 90(6):671-674.

Busi BR. Taste sensitivity to phenylthiourea among leprosy and filarial patients in coastal Andhra Pradesh. Acta Genet Med Gemellol (Roma) 1972; 21(4):332-336.

Cairncross S, Rajavel AR, Vanamail P, Subramaniam S, Paily KP, Ramaiah KD et al. Engineering, mosquitoes and filariasis: a case report. J Trop Med Hyg 1988; 91(3):101-

106.

Carmeliet P, Jain RK. 2011. Molecular mechanisms and clinical applica- tions of angiogenesis. Nature 473:298 –307.

Casley-Smith JR, Casley-Smith JR. The pathophysiology of lymphedema and the action of benzo-pyrones in reducing it. Lymphology 1988; 21(3): 190-194.

Casley-Smith JR, Jamal S, Caslcy-Smith J. Reduction of filaritic lymphoedema and elephantiasis by 5,6 benzo-alpha- pyrone (coumarin), and the effects of diethylcarbamazine (DEC). Ann Trop Med Parasitol 1993; 87(3):247-258.

Chakravertty RK, Dey KP, Joshi RD, Biswas G, Kumar A. Man vector contact in microfilaraemic and amicrofilaraemic human population-a case control study. J Commun Dis 1991; 23(2):123-127.

Chakravertty RK, Srivastav VK, Srivastava SP, Chand P, Rao CK. Prophylactic effect of diethylcarbamazine on Wuchereria bancrofti filariasis. J Commun Dis 1987; 19(2):128-135.

Chan MS, Srividya A, Norman RA, Pani SP, Ramaiah KD, Vanamail P et al. Epifil: a dynamic model of infection and disease in lymphatic filariasis. Am J Trop Med Hyg 1998; 59(4):606-614.

Chand G, Pandey GD, Tiwary RS. Prevalence of Wuchereria bancrofti infection among the tribals of Panna district of Madhya Pradesh. J Commun Dis 1996; 28(4):304-307.

Chand SK, Yadav RS, Sharma VP. Seasonality of indoor resting mosquitoes in a broken-forest ecosystem of north-western Orissa. Indian J Malariol 1993; 30(3):145-154.

Chandra G, Hati AK. Correlation between the preferred biting site of Culex quinquefasciatus and the region of the body affected by clinical filariasis. Ann Trop Med Parasilol 1993; 87(4):393-397.

Chandra G, Hati AK. Filariasis survey in a rural area of West Bengal. J Commun Dis 1996; 28(3):206-208.

Chandra G. Short report: peak period of filarial transmission. Am J Trop Med Hyg 1995; 53(4):378-379.

Chandra S, Chandra R, Katiyar JC, Govil P, George PA, Sen AB. Observations on filariasis in some villages around Lucknow, Uttar Pradesh. Indian J Med Res 1973;61(8):1127-1133.

Chandrasekharan A, Balaraman K, Rao CK. Decline in brugian filariasis in hill tribe settlements of Kerala. Indian J Med Res 1981; 73 Suppl:82-86.

Chandrasekharan A, Ghosh TK, Jain DC, Thomas TG. Relative role of different types of aquatic plants in the production of Mansonia. J Commun Dis 1988; 20(3):202-208.

Chandrasekharan A, Kochukrishna PK, Cherian C, Roychowdhury SP, Rao CK. Control of Brugia malayi filariasis with common salt medicated with DEC in some hill-tribe settlements of Kerala. Indian J Med Res 1984; 79:600-603.

Chari MV. Diagnosis and epidemiology of tropical eosinophilia--a review. Indian J Chest Dis 1971; 13(4):177-183.

Charters AID. Filariasis in immigrants in Western Australia, including a case of chyluria

recurring in seven pregnancies. Med J Aust 1970; 2(20):919-922.

Chaturvedi P, Harinath BC, Reddy MV, Advani B, Gawdi A, Alikhan A et al. Filarial antibody detection in suspected occult filariasis in children in an endemic area. J Trop Pediatr 1995; 41(4):243-245.

Chauhan PP, Pande BP. Morphological variations within the species Onchocerca armillata and O. gutturosa in buffaloes and cattle in India, with special reference to the male tail and cuticular ornamentation. J Helminthol 1978; 52(4):343-353.

Chhotray GP, Ranjit MR, Mohapatra M. Occurrence of asymptomatic microscopic haematuria in a filarial endemic area of Orissa, India. J Commun Dis 2000; 32(2):85-93.

Choi EH, Zimmerman PA, Foster CB, Zhu S, Kumaraswami V, Nutman TB et al. Genetic polymorphisms in molecules of innate immunity and susceptibility to infection with Wuchereria bancrofti in South India. Genes Immun 2001; 2(5):248-253.

Choudhry A, Guru PY, Saxena RP, Tandon A, Saxena KC. Enzyme-linked immunosorbent assay in the diagnosis of kala-azar in Bhadohi (Varanasi), India. Trans R Soc Trop Med Hyg 1990; 84(3):363-366.

Choudhury A, Misra KK, Majumdar G. Microfilariae from the black headed shrike. Trans R Soc Trop Med Hyg 1972; 66(4):671-672.

Chowdhury AB, Schiller EL. A survey of parasitic infections in a rural community near Calcutta. Am J Epidemiol 1968; 87(2):299-312.

Chowdhury AB. Profile of filariasis problem. J Indian Med Assoc 1971; 56(12):385-386.

Chowdhury AN. Penile perception of Koro patients. Acta Psychiatr Scand 1989; 80(2): 183-186.

Connor DH, George GH, Gibson DW. Pathologic changes of human onchocerciasis: implications for future research. Rev Infect Dis 1985; 7(6):809-819.

Connor DH, Palmieri JR, Gibson DW. Pathogenesis of lymphatic filariasis in man. Z Parasitenkd. 1986; 72:13–28. [PubMed: 3515791]

Croll NA, Ghadirian E, Sukul NC. Exposure and susceptibility in human helminthiasis. Trop Doct 1982; 12(3): 136-138.

Das BK, Sahoo PK, Ravindran B. A role for tumour necrosis factor-alpha in acute lymphatic filariasis. Parasite Immunol. 1996; 18:421–424. [PubMed: 9229396]

Das MK, Beuria MK, Dash AP. Immunoglobulin E and G4 antibodies to infective larvae in a Wuchereria bancrofti endemic population. Int Arch Allergy Immunol 1992; 99(1): 118-122.

Das PK, Manoharan A, Ramaiah KD, Balarajan K, Dhanda V. Cost analysis of blood surveys for the detection of microfilaria carriers in rural areas. Natl Med J India 1995; 8(3): 143-144.

Das PK, Manoharan A, Srividya A, Grenfell BT, Bundy DA, Vanamail P. Frequency distribution of Wuchereria bancrofti microfilariae in human populations and its relationships with age and sex. Parasitology 1990; 101 Pt 3:429-434.

Das PK, Manoharan A, Subramanian S, Ramaiah KD, Pani SP, Rajavcl AR et al. Bancroftian filariasis in Pondicherry, south India—epidemiological impact of recovery of

the vector population. Epidemiol Infect 1992; 108(3):483-493.

Das PK, Ramaiah KD, Vanamail P, Pani SP, Yuvaraj J, Balarajan K et a I. Placebo-controlled community trial of four cycles of single-dose diethylcarbamazine or ivermectin against Wuchereria bancrofti infection and transmission in India. Trans R Soc Trop Med Hyg 2001; 95(3):336-341.

Das PK, Sirvidya A, Vanamail P, Ramaiah KD, Pani SP, Michael E et al. Wuchereria bancrofti microfilaraemia in children in relation to parental infection status. Trans R Soc Trop Med Hyg 1997; 91(6):677-679.

Das PK, Srividya A, Pani SP, Ramaiah KD, Vanamail P, Dhanda V. Cumulative exposure and its relationship with chronic filarial disease in bancroftian filariasis. Southeast Asian J Trop Med Public Health 1994; 25(3):516-521.

Das SC, Nath DR, Bhuyan M, Das NG, Baruah I, Talukdar PK. Studies on malaria and filariasis vectors in Kamorta and Great Nicobar Islands. Indian J Malariol 1989; 26(3): 153-157.

Dash AP, Mahapatra N, Hazra RK, Acharya AS. Transmission dynamics of filariasis in Khurdha district of Orissa, India. Southeast Asian J Trop Med Public Health 1998; 29(1):137-140.

Date A, Neela P, Shastry JC. Mcmbranoproliferative glomerulonephritis in a tropical environment. Ann Trop Med Parasitol 1983; 77(3):279-285.

Date A, Vaska K, Vaska PH, Pandey AP, Kirubakaran MG, Shastry JC. Terminal infections in renal transplant patients in a tropical environment. Nephron 1982; 32(3):253-257.

Davies J, Spry CJ, Vijayaraghavan G, De Souza JA. A comparison of the clinical and cardiological features of endomyocardial disease in temperate and tropical regions. Postgrad Med J 1983; 59(689): 179-185.

De SK, Chandra G. Studies on the filariasis vector-Culex quinquefasciatus at Kanchrapara, west Bengal (India). Indian J Med Res 1994; 99:255-258.

Debrah AY, et al. 2006. Doxycycline reduces plasma VEGF-C/sVEGFR-3and improves pathology in lymphatic filariasis. PLoS Pathog. 2:e92 doi:10.1371/journal.ppat.0020092

Debrah AY, et al. 2009. Reduction in levels of plasma vascular endothelial growth factor-A and improvement in hydrocele patients by targeting en- dosymbiotic Wolbachia sp. in Wuchereria bancrofti with doxycycline. Am. J. Trop. Med. Hyg. 80:956 –963.

Debrah AY, Mand S, Specht S, et al.: Doxycycline reduces plasma VEGF-C/sVEGFR-3 and improves pathology in lymphatic filariasis. PLoS Pathog. Sep 2006;2:e92.

Deshmukh PG. On a new species of the genus Paronchocerca from rock pigeon Columba livia. Riv Parassitol 1969; 30(4):291-294.

Dhanda V, Das PK, Lai R, Srinivasan R, Ramaiah KD. Spread of lymphatic filariasis, re-emergence of leishmaniasis & threat of babesiosis in India. Indian J Med Res 1996; 103:46-54.

Dhar GM, Prasad BG, Bhatnagar JK, Maihur YD. A survey on rats, rat fleas, house-flies and mosquitoes in village Rahimabad, Lucknow district. Indian J Med Res 1972; 60(8): 1232-1241.

Diesfeld HJ, Dutta SN, Braun-Munzinger R. [Immundiagnostic studies on persons from a Wuchereria bancrofti endemic area]. Z Tropenmed Parasitol 1973; 24(4):439-446.

Dissanaike AS, Abeyewickreme W, Wijesundera MD, Weerasooriya MV, Ismail MM. Human dirofilariasis caused by Dirofilaria (Nochtiella) repens in Sri Lanka. Parassitologia 1997; 39(4):375-382.

Dixit V, Prasad GB. Seroprevalences of antibodies against thoracic proteins of three species of mosquito, among the residents of an area where filariasis is endemic: relationship with the mosquito densities. Ann Trop Med Parasitol 2001; 95(4):399-403.

Dondero TJ, Jr., Bhattacharya NC, Black HR, Chowdhury AB, Gubler DJ, Inui TS et al. Clinical manifestations of Bancroftian filariasis in a suburb of Calcutta, India. Am J Trop Med Hyg 1976; 25(l):64-73.

Dondero TJ, Jr., Menon VV. Clinical epidemiology of filariasis due to Brugia malayi on a rubber estate in West Malaysia. Southeast Asian J Trop Med Public Health 1972; 3(3):355-365.

Dorta DM, Vasuki V, Rajavel A. Evaluation of organophosphorus and synthetic pyrethroid insecticides against six vector mosquitoe species. Rev Saude Publica 1993;27(6):391-397.

Dreyer G, Addiss D, Dreyer P, Noroes J (2002) Basic lymphedema management: treatment and prevetion of problems associated with lymphatic filariasis. Hollis, NH: Hollis Publishing.

Dreyer G, Amaral F, Noroes J, Medeiros Z. Ultrasonographic evidence for stability of adult worm location in bancroftian filariasis. Trans R Soc Trop Med Hyg. 1994; 88:558. [PubMed: 7992338]

Dreyer G, Dreyer P, Piessens WF: Extralymphatic disease due to bancroftian filariasis. *Braz J Med Biol Res* 1999, 32:1467-1472.

Dreyer G, Medeiros Z, Netto MJ, Leal NC, de Castro LG, Piessens WF. Acute attacks in the extremities of persons living in an area endemic for bancroftian filariasis: differentiation of two syndromes. Trans R Soc Trop Med Hyg. 1999; 93:413–417. [PubMed: 10674092]

Dreyer G, Noroes J, Addiss D, Santos A, Medeiros Z, Figueredo-Silva J. Bancroftian filariasis in a paediatric population: an ultrasonographic study. Trans R Soc Trop Med Hyg. 1999; 93:633–636. [PubMed: 10717753]

Dreyer G, Noroes J, Figueredo-Silva J, Piessens WF. Pathogenesis of lymphatic disease in bancroftian filariasis: a clinical perspective. Parasitol Today. 2000; 16:544–548. [PubMed: 11121854]

Dreyer G, Noroes J, Figueredo-Silva J, Piessens WF: Pathogenesis Editorial. J Trop Med Hyg 1974; 77(5):93.

Dreyer G, Noroes J, Figueredo-Silva J, Piessens WF: Pathogenesis of lymphatic disease in bancroftian filariasis: A clinical perspective. Parasitology Today. 2000, 16 (12): 544-548. 10.1016/S0169-4758(00)01778-6.

Dreyer G, Ottesen EA, Galdino E, Andrade L, Rocha A, Medeiros Z, Moura I, Casimiro I, Beliz F, Coutinho A. Renal abnormalities in microfilaremic patients with Bancroftian filariasis. Am J Trop Med Hyg. 1992; 46:745–751. [PubMed: 1621900]

Dreyer G, Piessens WF: Worms and microorganisms can cause disease in residents of filariasis-endemic areas. In *Lymphatic Filariasis* Nutman TB: Imperial College Press; 2000:239-256.

Dreyer G: New insights into the natural history and pathology of bancroftian filariasis: Implications for clinical management and filariasis control programmes. *Trans R Soc Trop Med Hyg* 2000, 94:594-596.

Dutta A, Sukul NC. Filaricidal properties of a wild herb, Andrographis paniculata. J Helminthol 1982; 56(2):81-84.

Dutta P, Gogoi BK, Chelleng PK, Bhattacharyya DR, Khan SA, Goswami BK et al. Filariasis in the labour population of a tea estate in Upper Assam. Indian J Med Res 1995; 101:245-246.

Dutta SN, Chakrabarty BC, Mandal S. Electrophoretic pattern of serum proteins in filaria cases in coal mines area. J Commun Dis 1980; 12(2):78-81.

Dutta SN, Diesfeld H.T, Kirsten C. [Displacentar transfer of fluorescent antibodies from mothers to their newborns in a Wuchereria bancrofti endemic area in India (author's transl)]. Tropenmed Parasitol 1976; 27(4):479-482.

Dutta SN, Diesfeld HJ. Evidence of sex variations in microfilaraemia and fluorescent antibody titre level at puberty in a bancroftian filariasis endemic area. J Commun Dis 1994; 26(1):43-51.

Dutta SN, Diesfeld JH. Indirect immunofluorescence test against Dipetalonema viteae in detection of filariasis in Dhanbad coalmines area. Indian J Med Res 1978; 67:553-561.

Duvanel T, Auckenthaler R, Rohner P, Harms M, Saurat JH: Quantitative cultures of biopsy specimens from cutaneous cellulitis. *Arch Intern Med* 1989, 149(2):293-296.

Edeson JF.The epidemiology and treatment of infection due to Brugia malayi. Bull World Health Organ 1962; 27(4-5): 529- 541.

el-Sharkawy IM, Haseeb AN, Saleh WA. Serum levels of endothelin-1 (ET-1), interleukin-2 (IL-2) and amino-terminal propeptide type III procollagen (PIII NP) in patients with acute and chronic filariasis. J Egypt Soc Parasitol. 2001; 31:169–176. [PubMed: 12557940]

Evans, D.B., Gelband, H., Vlassoff, C., 1993. Social and economic factors and the control of lymphatic filariasis: a review. Acta Trop. 53, 1–26.

factors between infected and uninfected respondents in a Malay community.

Figueredo-Silva J, Noroes J, Cedenho A, Dreyer G. 2002. The histopathology of bancroftian filariasis revisited: the role of the adult worm in the lymphatic vessel disease. Ann. Trop. Med. Parasitol. 96:531–541. 11.

Figueredo-Silva J, Noroes J, Cedenho A, Dreyer G. The histopathology of bancroftian filariasis revisited: the role of the adult worm in the lymphatic-vessel disease. Ann Trop Med Parasitol. 2002; 96:531–541. [PubMed: 12396316]

Filariasis Elimination in India: Hope for the Next Millennium. Department of Public Health and Preventive Medicine, Government of Tamil Nadu and Vector Control Research Centre, Pondicherry, p.1, 1999. Filariasis. Q Med Rev 1974; 25(2):8-23.

Freedman DO, de Almeida Filho PJ, Besh S, Maia e Silva MC, Braga C, Maciel A. Lymphoscintigraphic analysis of lymphatic abnormalities in symptomatic and asymptomatic human filariasis. J Infect Dis. 1994; 170:927–933. [PubMed: 7523538]

Freedman DO, de Almeido Filho PJ, Besh S, Maia e Silva MC, Braga C, Maciel A, Furtado AF. Abnormal lymphatic function in presymptomatic bancroftian filariasis. J Infect Dis. 1995; 171:997– 1001. [PubMed: 7706830]

Gaur SD, Marwah SM. Role of health education in filaria control. Indian J Public Health 1967; 11(3):131-132.

Gelband H. Diethylcarbamazine salt in the control of lymphatic filariasis. Am J Trop Med Hyg 1994; 50(6):655-662.

George GH, Palmieri JR, Connor DH. The onchocerca! nodule: interrelationship of adult worms and blood vessels. Am J Trop Med Hyg 1985; 34(6): 1144-1148.

Ghosh SK, Yadav RS. Naturally acquired concomitant infections of bancroftian filariasis and human Plasmodia in Orissa. Indian J Malariol 1995; 32(l):32-36.

Ghosh TK. Problem in filariasis control. J Commun Dis 1985; 17(1 Suppl):110-112.

Gopinath R, Hanna LE, Kumaraswami V, Perumal V, Kavitha V, Vijayasekaran V et al. Perturbations in eosinophil homeostasis following treatment of lymphatic filariasis. Infect Immun 2000; 68(l):93-99.

Govt. of India, Ministry of Health and Family Welfare: National vector borne diseases control programme; Problems and elimination of lymphatic filariasis in India. 2005.Available from: http://www.namp.gov.in/filariasis.html . [cited on 2010 Jan 12].

Gowda NN, Vijayan VA. Indoor resting density, survival rate and host preference of Culex quinquefasciatus say (Diptera: Culicidae) in Mysore City. J Commun Dis 1992; 24(l):20-28.

Grace A, Grace F, Warren S: The parallel incidence of filaria bancrofti and the B-Haemolytic streptococcus in certain tropical countries. *Am J Trop Med Hyg* 1932, 12(6):493-509.

Grenfell BT, Das PK, Rajagopalan PK, Bundy DA. Frequency distribution of lymphatic filariasis microfilariae in human populations: population processes and statistical estimation. Parasitology 1990; 101 Pt 3:417-427.

Gubler DJ, Bhattacharya NC. A quantitative approach to the study of Bancroftian filariasis. Am J Trop Med Hyg 1974; 23(6): 1027-1036.

Gupta KK. Prevalence of filariasis among naval community residing in Navy Nagar, Bombay. Indian J Public Health 1988; 32(3): 160-161.

Gupta S, Srivastava JK, Ray S, Chandra R, Srivastava VK, Katiyar JC. Evaluation of enzyme-linked immunosorbent assay in the diagnosis of kala-azar in Malda district (West Bengal). Indian J Med Res 1993; 97:242-246.

Gupta SC, Misra V, Gupta AK. Cavernous lymphangioma in the vulva. Trop Doct 1998; 28(1):52.

Gyapong JO, Gyapong M, Adjei S: The epidemiology of acute adenolymphangitis due to lymphatic filariasis in northern Ghana. Am J Trop Med Hyg. 1996, 54 (6): 591-595.

Gyorkos TW, Sukul NC, DasDal A. Filariasis and ABO blood group status: a critical appraisal. Trans R Soc Trop Med Hyg 1983; 77(4):564-565.

Hamilton RG, Hussain R, Oltesen EA. Immunoradiometric assay for detection of filarial antigens in human serum. J Immunol 1984; 133(4):2237-2242.

Hartgers FC, et al. 2008. Enhanced Toll-like receptor responsiveness associated with mitogen-activated protein kinase activation in Plasmo-dium falciparum-infected children. Infect. Immun. 76:5149 –5157.

Hati AK, Chandra G, Bhattacharyya A, Biswas D, Chatterjee KK, Dwibedi HN. Annual transmission potential of bancroftian filariasis in an urban and a rural area of West Bengal, India. Am J Trop Med Hyg 1989; 40(4):365-367.

Hawking F, Pattanayak S, Sharma HL. The periodicity of microfilariae. XI. The effect of body temperature and other stimuli upon the cycles of Wuchereria bancrofti, Brugia malayi, B. ceylonensis and Dirofilaria repens. Trans R Soc Trop Med Hyg 1966; 60(4):497-513.

Hawking F. Filariasis in India. Prog Drug Res 1974; 18:173-190.

Hinz E. [The spread of helminthiases in man in south Asia. A medical-geographical review]. Munch Med Wochenschr 1972; 114(40): 1705-1712.

HiseAG,etal.2007. Innate immune responses to endosymbiotic Wolbachia bacteria in Brugia malayi and Onchocerca volvulus are dependent on TLR2, TLR6, MyD88, and Mal, but not TLR4, TRIF, or TRAM. J. Immunol. 178:1068 –1076.

Honorati MC, Cattini L, Facchini A. 2004. IL-17, IL-1a and TNF-a stimulate VEGF production by dedifferentiated chondrocytes. Osteoarthritis Cartilage 12:683– 691.

Horton J, Witt C, Ottesen EA, Lazdins JK, Addiss DG, Awadzi K et al. An analysis of the safety of the single dose, two drug regimens used in programmes to eliminate lymphatic filariasis. Parasitology 2000; 121 Suppl:S147-S160.

Hotez, P.J., Fenwick, A., Savioli, L., Molyneux, D.H., 2009. Rescuing the bottom billion through control of neglected tropical diseases. Lancet 373, 1570–1575.

Hotez, P.J., Kamath, A., 2009. Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden. PLoS. Negl. Trop. Dis. 3, e412.

Hoti SL, Vasuki V, Lizotte MW, Patra KP, Ravi G, Vanamail P et al. Detection of Brugia malayi in laboratory and wild-caught Mansonioides mosquitoes (Diptera: Culicidae) using Hha IPCR assay. Bull Entomol Res 2001; 91(2):87-92.

Houston R. Salt fortified with diethylcarbamazine (DEC) as an effective intervention for lymphatic filariasis, with lessons learned from salt iodization programmes. Parasitology 2000; 121 Suppl:S161-S173.

http://www.who.int/mediacentre/factsheets/fs102/en/

Hussain R, Hamilton RG, Kumaraswami V, Adkinson NF, Jr., Ottesen EA. IgE responses in human filariasis. I. Quantitation of filaria-specific IgE. J Immunol 1981; 127(4): 1623-1629.

Hyma B, Ramesh A, Gunasekaran K. Lymphatic filariasis in Madras, India. Soc Sci Med 1989; 29(8):983-990.

Indrayan A, Srivastava RN, Bagchi SC. Mathematical models in the assessment of infective force in filariasi. Indian J Med Res 1970; 58(8):1100-1103.

Jain DC, Chandrasekharan A, Sethumadhavan KV, Johny VM, Cherian C, Ghosh TK. Epidemiology of brugian filariasis in a rural community of Kerala State. J Commun Dis 1989; 21(l):27-33.

Jain DC, Menon PK, Sethumadhavan KV, Johney VM, Ghosh TK. Epidemiology of bancroftian filariasis in a semi-urban community of Kerala State. J Commun Dis 1989;21(4):265-271.

Jain DC, Ravindranathan TC, Ghosh TK, Pillai KB, Rao CK. Prophylactic effect of diethylcarbamazine on W. bancrofti filariasis. J Commun Dis 1987; 19(4):317-321.

Jain DC, Sunny PJ, Prakash KS. Role of health education on voluntary community participation in anti- larval measures through clearance of aquatic plants in a Brugian endemic area. J Commun Dis 1986; 18(l):54-56.

Jain S, Rani S, Jain SK. Unsuspected filariasis coexisting with leishmaniasis in a splenic aspirate. J Assoc Physicians India 1998; 46(2):238-239.

Jain SP, Singh J. Traditional medicinal practice among the tribal people of raigarh (Chattishgarh), India. Ind J Nat Product Resources 2010; 1: 109-115.

Jamal S, Casley-Smith JR, Casley-Smith JR. The effects of 5,6 benzo-[a]-pyrone (coumarin) and DEC on filaritic lymphoedema and elephantiasis in India. Preliminary results. Ann Trop Med Parasitol 1989; 83(3):287-290.

Jayaraman KS. Indian health planners shun insecticides in disease control. Nature 1987; 329(6140):572.

Jayasrce M, Sabesan S, Kuriakose KM, Panicker KN. Giant gourami (Osphronemus goramy: Anabantoidei) as a potential agent for control of weeds, the breeding source for the vectors of Brugia malayi. Indian J Med Res 1989; 89:110-113.

Joseph A. "Studies on the pattern of distribution of filaria carriers in a sea port of Kerala". Indian J Public Health 1971; 15(3):103-108.

Joseph G, Prasad BG. An epidemiological study of filariasis in the coastal bell of Kerala State. Indian J Med Res 1967; 55(11):1259-1272.

Kabatereine NB, Malecela M, Lado M, Zaramba S, Amiel O, Kolaczinski JH. How to (or not to) integrate vertical programmes for the control of major neglected tropical diseases in sub-Saharan Africa. PLoS Negl Trop Dis 2010; 4(6): e755.

Kanda K: The quality of life among lymphedema patients due to lymphatic filariasis in three rural towns in Haiti. 2004, Tampa, FL: University of South Florida

Kanetkar AV, Deshmukh SM, Pradhan RS, Kelkar MD, Sen PK. Lymphangiographic patterns in filarial oedema of lower limbs. Clin Radiol 1966; 17(3):258-263.

Kanhekar LJ, Patnaik SK, Rao PK, Narayana MV, Raina VK, Kumar A. Some aspects of the bionomics of Culex quinquefasciatus (Diptera: Culicidae) in Rajahmundry town, Andhra Pradesh. J Commun Dis 1994; 26(4):226-230.

Kapil A. Laboratory diagnosis of lymphatic filariasis. Indian J Pediatr 1989; 56(3):314-

317.

Kar I, Eapen A, Ravindran KJ, Chandrahas RK, Appavoo NC, Sadanand AV et al. Field evaluation of Bacillus sphaericus, H5a5b and B. thuringiensis var. israelensis, H-14 against the Bancroftian filariasis vector Culex quinquefasciatus, Say in Chennai, India. Indian J Malariol 1997; 34(l):25-36.

Kar SK, Mania J, Kar PK. Clinical filarial disease in two ethnic endemic communities of Orissa, India. J Trop Med Hyg 1993; 96(5):311-316.

Kar SK, Mania J, Kar PK. Prevalence of lymphatic nodule in a bancroftian endemic population. Acta Trop 1993; 55(l-2):53-60.

Kar SK, Mania J. Tropical pulmonary eosinophilia in an Orissa village. Natl Med J India 1993; 6(2):64-67.

Kar SK, Patnaik S, Mania J, Kumaraswami V. Ivermectin in the treatment of bancroftian filarial infection in Orissa, India. Southeast Asian J Trop Med Public Health 1993; 24(l):80-86.

Kar SK. Epidemiological study on lymphatic filariasis in an endemic zone of Orissa. J Commun Dis 1986; 18(4):312.

Karnaukhov VK. [Imported exotic diseases encountered in the practice of general practitioners and parasitologists]. Ter Arkh 1983; 55(5): 128-130.

Kaul SM, Raina VK, Joshi RD, Koya CN, Kumar A, Verghese T. Efficacy of diethylcarbamazine medicated salt in interrupting Brugia malayi transmission in hill tribe settlements in Kerala State. J Commun Dis 1992; 24(1): 16-19.

Khajuria RR. Incidence and control of parafilariasis in bovines in Jammu Province. Indian Vet J 1966; 43(8):749-750.

Khan AM, Dutta P, Khan SA, Baruah NK, Sarma CK, Mahanta J. Prevalence of bancroftian filariasis in a foot-hill tea garden of upper Assam. J Commun Dis 1999; 31(2): 145-146.

Khan AM, Dutta P, Khan SA, Baruah NK, Sharma CK, Mahanta J. Bancroftian filariasis in a weaving community of lower Assam. J Commun Dis 1999; 31(l):61-62.

Khan AM, Dutta P, Khan SA, Mohapatra PK, Baruah NK, Sharma CK et al Lymphatic filariasis in two distinct communities of upper Assam. J Commun Dis 1999; 31(2):101-106.

King CL, Nutman TB. 1991. Regulation of the immune response in lymphatic filariasis and onchocerciasis. Immunol. Today 12:A54 –A58.

Kirkwood, B., Smith, P., Marshall, T., Prost, A., 1983. Relationships between mortality, visual acuity and microfilarial load in the area of the Onchocerciasis Control Programme. Trans. R. Soc. Trop. Med. Hyg. 77, 862–868.

Kopp E, Medzhitov R. 2003. Recognition of microbial infection by Toll-like receptors. Curr. Opin. Immunol. 15:396 – 401.

Koya SM, Kumar S, Regu K, Sharma RC. Early development of acute filariasis in a migrant from non-endemic to hyper endemic area: a case report. J Commun Dis 1998; 30(2):117-120.

Krishnamoorthy K, Rajendran G, Panicker KN. Aquatic vegetation and their natural hospitability to the immatures of Mansonia mosquitos, the vectors of Brugia malayi in Shertallai, Kerala, India. Southeast Asian J Trop Med Public Health 1994; 25(4):760-765.

Krishnamoorthy K, Ramu K, Srividya A, Appavoo NC, Saxena NB, Lai S et al. Cost of mass annual single dose diethylcarbamazine distribution for the large scale control of lymphatic filariasis. Indian J Med Res 2000; 111:81-89.

Krishnamoorthy K, Sabesan S, Vanamail P, Panicker KN. Influence of diethylcarbamazine on the patent period of infection in periodic Brugia malayi. Indian J Med Res 1991; 93:240-244.

Krishnamoorthy K. Estimated costs of acute adenolymphangitis to patients with chronic manifestations of bancroftian filariasis in India. Indian J Public Health 1999;43(2):58-63.

Kumar A, Chand SK. Prevalence of Wuchereria bancrofti infection in some coastal villages of Ganjam, Orissa. J Commun Dis 1990; 22(3):209-212.

Kumar A, Dash AP, Mansing GD. Prevalence of filariasis in rural Puri, Orissa. J Commun Dis 1994; 26(4):215-220.

Kumar A. Do offspring of filarial infected mothers have a greater risk of becoming microfilaraemic? Acta Trop 1997; 64(3-4):219-223.

Kumar A. Household characteristics and peoples' perception about filariasis in Khurda district Orissa. J Commun Dis 1999; 31(2): 147-150.

Kumar A. Human filariasis: infection rate as the uniform measurable criterion for filarial endemicity. J Commun Dis 1996; 28(3):163-167.

Kumar H, Santhaman S. Dot-ELISA in detection of filarial antibodies: comparison with standard ELISA. J Hyg Epidemiol Microbiol Immunol 1990; 34(2):213-218.

Kumar NP, Sabesan S, Panicker KN. Biting rhythm of the vectors of Malayan filariasis, Mansonia annulifera, M. uniformls & M. indiana in Shertallai (Kerala state), India. Indian J Med Res 1989; 89:52-55.

Kumar NP, Sabesan S, Panicker KN. Susceptibility status of Mansonia annulifera to Brugia malayi parasites in Cherthala, Alappuzha district, Kerala. Indian J Exp Biol 1998; 36(8):829-831.

Kumar NP, Sabesan S, Panicker KN. The resting and house frequenting behavior of Mansonia annulifera. Ma. uniformis and Ma. indiana, the vectors of Malayan filariasis in Kerala State, India. Southeast Asian J Trop Med Public Health 1992; 23(2):324-327.

Kumaraswami V, Ottesen EA, Vijayasekaran V, Devi U, Swaminathan M, Aziz MA et al. Ivermectin for the treatment of Wuchereria bancrofti filariasis. Efficacy and adverse reactions. JAMA 1988; 259(21):3150-3153.

Kutty VR, Abraham S, Kartha CC. Geographical distribution of endomyocardial fibrosis in south Kerala. Int J Epidemiol 1996; 25(6):1202-1207.

Lahiri VL. Microfilariae in nipple secretion. Acta Cytol 1975; 19(2):154.

Lakshmi A. A statistical approach to monitor ongoing intervention for control of lymphatic filariasis. J Commun Dis 2000; 32(1):10-16.

Lal RB, Dhawan RR, Ramzy RM, Farris RM, Gad AA. C-reactive protein in patients with lymphatic filariasis: increased expression on lymphocytes in chronic lymphatic obstruction. J Clin Immunol. 1991; 11:46–53. [PubMed: 2022721]

Lal RB, Ottesen EA. 1988. Enhanced diagnostic specificity in human filariasis by IgG4 antibody assessment. J. Infect. Dis. 158:1034 –1037.

Lalitha P, Ravichandran M, Suba S, Kaliraj P, Narayanan RB, Jayaraman K. Quantitative assessment of circulating antigens in human lymphatic filariasis: a field evaluation of monoclonal antibody-based ELISA using blood collected on filter strips. Trop Med Int Health 1998; 3(l):41-45.

Lammie PJ, Cuenco KT, Punkosdy GA: The pathogenesis of filarial lymphedema: Is it the worm or is it the host? *Ann NY Acad Sci* 2002, 979(1):131-142.

Lang AP, Luchsinger IS, Rawling EG. Filariasis of the breast. Arch Pathol Lab Med 1987; lll(8):757-759.

Limbos P. Etiology of eosinophilia in Europeans returning from central Africa. Indian J Chest Dis 1971; 13(4): 170-176.

Little, M.P., Breitling, L.P., Basanez, M.G., Alley, E.S., Boatin, B.A., 2004. Association between microfilarial load and excess mortality in onchocerciasis: an epidemi- ological study. Lancet 363, 1514–1521.

Lohela M, Bry M, Tammela T, Alitalo K. 2009. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. Curr. Opin. Cell Biol.21:154 –165.

Lopez N, de Boissieu D. [Iconographic rubric. A case with tropical pulmonary eosinophilia]. Arch Fr Pediatr 1992; 49(8):733-734.

Ludlam KW, Jachowski LA, Jr., Otto GF. Potential vectors of Dirofilaria immitis. J Am Vet Med Assoc 1970; 157(10):1354-1359.

Lunde MN, Paranjape R, Lawley T.I, Ottesen EA. Filarial antigen in circulating immune complexes from patients with Wuchereria bancrofti filariasis. Am J Trop Med Hyg 1988; 38(2):366-371.

Macdonald WW. Control of Culex quinquefasciatus in Myanmar (Burma) and India: 1960- 1990. Ann Trop Med Parasitol 1991; 85(1):165-172.

Mahanta B, Handique R, Dutta P, Narain K, Mahanta J. Temporal variations in biting density and rhythm of Culex quinquefasciatus in tea agro-ecosystem of Assam, India. Southeast Asian J Trop Med Public Health 1999; 30(4):804-809.

Mandal NN, Das MK. A four year follow up study of filaria specific IgE response in individuals with hydrocele. Indian J Med Res 2000; 112:203-205.

Mani TR, Reuben R, Akiyama J. Field efficacy of "Mosbar" mosquito repellent soap against vectors of bancroftian filariasis and Japanese encephalitis in southern India. J Am Mosq Control Assoc 1991; 7(4):565-568.

Manoharan A, Das PK, Keerthiseelan VB, Ramaiah KD. Trend of Wuchereria bancrofti infection in Pondicherry urban agglomeration after the withdrawal of a five year vector control programme. J Commun Dis 1997; 29(3):255-261.

Manoharan A, Ramaiah KD, Subramanian S, Das PK. Spatial and temporal variations in prevalence, intensity and aggregation of microfilaria in the human host. Southeast Asian J

Trop Med Public Health 1993; 24(2);327-332.

Marwah SM, Rao NS, Gaur SD. A study of filariasis in an aggregated community of Banaras Hindu University township. J Indian Med Assoc 1973; 60(2):33-39.

mass drug administration during the programme to eliminate lymphatic filariasis in Orissa, India. Trop Med Int Health 9:702–709.

Mayrovitz HN. The standard of care for lymphedema: current concepts and physiological considerations.*Lymphat Res Biol*. 2009. 7(2):101-8.

McCarthy J. Diagnosis of lymphatic filarial infection. In: Nutman TB. Lymphatic filariasis. London: Imperial College Press; 2000,p. 127-141

McKeehan FR. Tropical eosinophilia in an American college: a report of two cases. J Am Coll Health Assoc 1975; 24(2):92-94.

McSorley HJ, Maizels RM. Helminth infections and host immune regulation. *Clin. Microbiol. Rev* 2012; 25:585–608.

Melrose WD: Lymphatic filariasis: New insights into an old disease.*Int J Parasitol* 2002, 32(8):947-960.

Miall WE, Bras G. Heart disease in the tropics. Br Med Bull 1972; 28(l):79-86.

Michael E, Bundy DA, Grenfell BT. Re-assessing the global prevalence and distribution of lymphatic filariasis. Parasitology 1996; 112 (Pt 4):409-428.

Mishra SS, Dwivedi MP. An epidemiological study of filariasis in Rewa town (M.P.). Indian J Public Health 1979; 23(1):7-16.

Misra N, Sharma M, Raj K, Dangi A, Srivastava S, Misra-Bhattacharya S. Chemical constituents and antifilarial activity of *Lantana camara* against human lymphatic filariid *Brugia malayi* and rodent filariid *Acanthocheilonema viteae* maintained in rodent hosts. *Parasitol. Res*. 100(3), 439–448 (2007).

Mohan G, Chaturvedi S, Misra PK. Microfilaria in a fine needle aspirate of primary solid malignant tumor of the maxillary antrum. A case report. Acta Cytol 1998; 42(3):772-774.

Mukherjee SK, Mukherjee AK, Sen JK. Letter: Filariasis with recurrent hematuria. JAMA 1974; 230(9):1254-1255.

Muller R, Hajdu SI, Brennan MF. Lymphangiosarcoma associated with chronic filarial lymphedema. Cancer 1987; 59(1):179-183.

Nandi NC. Brugia-type microfilariae in the Madras tree shrew Anathana ellioti (Waterhouse). J Helminthol 1982; 56(2):93-94.

Narain JP, Dash AP, Parnell B, Bhattacharya SK, Barua S, Bhatia R, et al. Elimination of neglected tropical diseases in the South- East Asia Region of the World Health Organization. Bull World Health Organ 2010; 88(3): 206-210.

Narasimham MV, Krishna RC, Rao CK. Some clinical aspects of bancroftian filariasis in East Godavari district, Andhra Pradesh. Indian J Med Res 1983; 78:631-635.

Narasimham MV, Sharma SP, Sundaram RM, Reddy GS, Raina VK, Sambasivam V et al. Control of bancroftian filariasis by diethylcarbamazine medicated common salt in

Karaikal, Pondicherry, India. J Commun Dis 1989; 21(3):157-170.

Narasimham MV, Venkatanarayana M, Rao PK, Brahmam RK, Krishna RC, Rai Chowdhuri AN et al. Voluntary community participation in the control of vector borne diseases-filariasis. I Commun Dis 1983; 15(2): 106-110.

Narushima M, Mihara M, Yamamoto Y, Iida T, Koshima I, Mundinger GS. The intravascular stenting method for treatment of extremity lymphedema with multiconfiguration lymphaticovenous anastomoses.*Plast Reconstr Surg*. 2010 Mar. 125(3):935-43.

Norman G, Joseph A, Theodore A, Maruthamuthu M. Community-based teaching of tropical diseases: an experience with filariasis. Trop Doct 1999; 29(2):86-89.

Norman RA, Chan MS, Srividya A, Pani SP, Ramaiah KD, Vanamail P et al. EPIFIL: the development of an age-structured model for describing the transmission dynamics and control of lymphatic filariasis. Epidemiol Infect 2000; 124(3):529-541.

Noroes J, Addiss D, Amaral F, Coutinho A, Medeiros Z, Dreyer G. Occurrence of living adult Wuchereria bancrofti in the scrotal area of men with microfilaraemia. Trans R Soc Trop Med Hyg. 1996; 90:55–56. [PubMed: 8730313]

Noroes J, Addiss D, Santos A, Medeiros Z, Coutinho A, Dreyer G. Ultrasonographic evidence of abnormal lymphatic vessels in young men with adult Wuchereria bancrofti infection in the scrotal area. J Urol. 1996; 156:409–412. [PubMed: 8683691]

Nutman TB, Kumaraswami V. Regulation of the immune response in lymphatic filariasis: perspectives on acute and chronic infection with Wuchereria bancrofti in South India. Parasite Immunol 2001; 23(7):389-399.

O'Neill LA, Bowie AG. 2007. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat. Rev. Immunol. 7:353–364.

O'NeillLA.2008.When signaling pathways collide:positive and negative regulation of Toll-like receptor signal transduction. Immunity 29:12–20.

Okon OE, Iboh CI, Opara KN. Bancroftian filariasis among the Mbembe people of Cross River state, Nigeria. J Vector Borne Dis 2010; 47(2): 91-96.

Olszewski WL, Jamal S, Manokaran G, Pani S, Kumaraswami V, Kubicka U, Lukomska B, Dworczynski A, Swoboda E, Meisel-Mikolajczyk F. Bacteriologic studies of skin, tissue fluid, lymph, and lymph nodes in patients with filarial lymphedema. Am J Trop Med Hyg. 1997; 57:7– 15. [PubMed: 9242310].

Olszewski WL, Jamal S: Skin bacterial factor in progression of filarial lymphedema. *Lymphology* 1994, 27:148-149.

Omar MS, Sheikha AK, Al Amari OM, Abdalla SE, Musa RA. Field evaluation of two diagnostic antigen tests for Wuchereria bancrofti infection among Indian expatriates in Saudi Arabia. Southeast Asian J Trop Med Public Health 2000; 31(2):415-418.

Omar MS. A survey of bancroftian filariasis among South-East Asian expatriate workers in Saudi Arabia. Trop Med Int Health 1996; 1(2):155-160.

Ong RK, Doyle RL. Tropical pulmonary eosinophilia. Chest 1998; 113(6):1673-1679.

Ottesen EA, Neva FA, Paranjape RS, Tripathy SP, Thiruvengadam KV, Beaven MA. Specific allergic sensitsation to filarial antigens in tropical eosinophilia syndrome. Lancet

1979; 1(8127): 1158-1161.

Ottesen EA, Nutman TB. Tropical pulmonary eosinophilia. Annu Rev Med. 1992; 43:417–424. [PubMed: 1580599]

Ottesen EA: Efficacy of diethylcarbamazine in eradicating infection with lymphatic-dwelling filariae in humans. *Rev Infect Dis* 1985, 7(3):341-356.

Padigel UM, Reddy MV, Alikhan A, Harinath BC. Immunomonitoring of filarial patients during DEC therapy in an endemic area: a seven-year follow-up. J Trop Med Hyg 1995; 98(1):52-56.

Pandit CG. Communicable diseases in twentieth-century India. Am J Trop Med Hyg 1970; 19(3):375-382.

Pani SP, Balakrishnan N, Srividya A, Bundy DA, Grenfell BT. Clinical epidemiology of bancroftian filariasis: effect of age and gender. Trans R Soc Trop Med Hyg 1991; 85(2):260-264.

Pani SP, Hoti SL, Elango A, Yuvaraj J, Lall R, Ramaiah KD. Evaluation of the ICT whole blood antigen card test to detect infection due to nocturnally periodic Wuchereria bancrofti in South India. Trop Med Int Health 2000; 5(5):359-363.

Pani SP, Krishnamoorthy K, Rao AS, Prathiba J. Clinical manifestations in malayan filariasis infection with special reference to lymphoedema grading. Indian J Med Res 1990; 91:200-207.

Pani SP, Srividya A, Krishnamoorthy K, Das PK, Dhanda V. Rapid assessment procedures (RAP) for lymphatic filariasis. Natl Med J India 1997; 10(1): 19-22.

Pani SP, Srividya A. Clinical manifestations of bancroftian filariasis with special reference to lymphoedema grading. Indian J Med Res. 1995; 102:114–118. [PubMed: 8543349]

Pani SP, Srividya A. Clinical manifestations of bancroftian filariasis with special reference to lymphoedema grading. Indian J Med Res 1995; 102:114-118.

Pani SP, Subramanyam Reddy G, Das LK, Vanamail P, Hoti SL, Ramesh J, et al. Tolerability and efficacy of single dose albendazole, diethylcarbamazine citrate (DEC) or co-administration of albendazole with DEC in the clearance of Wuchereria bancrofti in asymptomatic microfilaraemic volunteers in Pondicherry, South India: A hospital-based study. Filaria J 2002;1:1.

Pani SP, Vanamail P, Srividya A, Das PK, Dhanda V. Micro-spatial variation in filarial disease and risk of developing disease associated with microfilaremia in urban situation. Southeast Asian J Trop Med Public Health 1994; 25(4):719-723.

Panicker KN, Arunachalam N, Kumar NP, Prathibha J, Sabesan S. Efficacy of diethylcarbamazine-medicated salt for microfilaraemia of Brugia malayi. Natl Med J India 1997; 10(6):275-276.

Panicker KN, Dhanda V. Community participation in the control of filariasis. World Health Forum 1992; 13(2-3):177-181.

Panicker KN, Jayasree M, Krishnamoorthy K. A cost benefit analysis of fish culture strategy towards the control of mansonioides in Shertallai, Kerala state. Indian J Med Res 1992; 95:157-160.

Panicker KN, Krishnamoorthy K, Sabesan S, Prathiba J, Abidha. Comparison of effects of

mass annual and biannual single dose therapy with diethylcarbamazine for the control of Malayan filariasis. Southeast Asian J Trop Med Public Health 1991; 22(3):402-411.

Panicker KN, Pani SP, Sabesan S, Krishnamoorthy K. Choice & integration of different approaches to case detection with special reference to brugian filariasis in south India. Indian J Med Res 1990; 91:282-288.

Partono F. The spectrum of disease in lymphatic filariasis. Ciba Found Symp. 1987; 127:15–31. [PubMed: 3297555]

Patel S, Rai RN, Mishra RN. Implementation of revised strategy of filaria control-baseline clinico- parasitological survey. J Commun Dis 2000; 32(2):77-83.

Patnaik B. Studies on stephanofilariasis in Orissa. 3. Life cycle of S. assamensis Pande, 1936. ZTropenmed Parasitol 1973; 24(4):457-466.

Patnaik SK, Narasimham MV, Ghosh TK, Rao CK, Kaur R, Narayana MV. Feasibility of controlling lymphatic filarial diseases through primary health care system. J Commun Dis 1989; 21(2): 107-110.

Patnaik SK, Rao PK, Narayana MV, Kanhekar LJ, Raina VK, Kumar A. Rural community perception and prevention practice towards filariasis in East Godavari District of Andhra Pradesh. J Commun Dis 1990; 22(4):285-286.

Pawar RG, Mittal MC. A study of periodicity of microfilaria bancrofti infections in Jamnagar. Indian J Med Sci 1967; 21:595-596.

Pawar RG, Mittal MC. Filariasis in Jamnagar. Indian J Med Res 1968; 56(3):370-376.

Pereira De Godoy JM, Amador Franco Brigidio P, Buzato E, Fátima Guerreiro De Godoy M. Intensive outpatient treatment of elephantiasis. *Int Angiol*. 2012 Oct. 31(5):494-9.

Pfarr KM, Debrah AY, Specht S, Hoerauf A. 2009. Filariasis and lymphoedema. Parasite Immunol. 31:664 – 672.

Pion, S.D., Kamgno, J., Demanga, N., Boussinesq, M., 2002. Excess mortality associated with blindness in the onchocerciasis focus of the Mbam Valley, Cameroon. Ann.Trop. Med. Parasitol. 96, 181–189.

Prakash A, Pandit PN, Sharma LK. Filarial breast abscess. Int Surg 1974; 59(2):112-113. 43:252–263.

Prakash A, Mohapatra PK, Das HK, Sharma RK, Mahanta J. Bancroftian filariasis in Namrup tea estate, district Dibrugarh, Assam. Indian J Public Health 1998; 42(4): 103-7, 112.

Prasad BG. Epidemiological research and its impact on health and medical care. Indian J Med Res 1972; 60(4):501-511.

Prasad MP, Rajendran G, Sabesan S, Kalyanasundaram M. Field evaluation of biodegradable controlled release formulation of fenthion against Mansonia mosquitos. Southeast Asian J Trop Med Public Health 1997; 28(1):208-211.

Prasad RN, Das MK, Sharma T, Dutta GD. Prevalence of filariasis in rural areas of Shahjahanpur district (Uttar Pradesh). Indian J Med Res 1993; 97:112-114.

Rahman N, Lim BH, Khairul AA, Shenoy RK, Kumaraswami V, Lokman HS et al. A

recombinant antigen-based IgG4 ELISA for the specific and sensitive detection of Brugia malayi infection. Trans R Soc Trop Med Hyg 2001; 95(3):280-284.

Rahman NM, Bhattacharyya MN. A preliminary survey of filarial infection in a group of labour population at Maijan tea estate in Lakhimpur district, Assam. J Indian Med Assoc 1971; 56(12):363-366.

Raina VK, Joshi MC, Joshi RD, Singh S, Yadava RL, Kumar A. Filaria situation in the Union Territory of Dadra & Nagar Haveli. J Commun Dis 1991; 23(3):182-184.

Raina VK, Joshi MC, Singh S, Joshi RD, Bhattacharjee KK, Kumar A et al. Epidemiology of Brugia malayi infection and its co-existence with Wuchereria bancrofti in and around Sillaberia PHC, District Midnapur, West Bengal. J Commun Dis 1990; 22(3):205-208.

Raina VK, Kosare GJ, Ras M, Rao CK. Impact of diethylcarbamazine treatment of selective microfilaria carriers and filarial cases under rural filaria control project in Valsad district, Gujarat. J Commun Dis 1984; 16(3):247-249.

Raina VK, Rao CK, Ahmad MB. Status of lymphatic filariasis transmission in Srinagar and Baramulla towns-Jammu and Kashmir state. J Commun Dis 1985; 17(l):92-93.

Raina VK, Tripathi VC, Ram R, Kumar A, Verghese T. Status of lymphatic filariasis in some select slum clusters of Delhi. J Commun Dis 1992; 24(2):92-96.

Rajagopalan PK, Bai MG, Arunachalam N. Age determination of man-biting population of Culex pipiens fatigans with particular reference to transmission of Wuchereria bancrofti in Pondicherry. Indian J Med Res 1981; 73:739-745.

Rajagopalan PK, Das PK, Pani SP, Mariappan T, Rajavel AR, Ramaiah KD et al. Evaluation of integrated vector control measures on filariasis transmission in Pondicherry. Indian J Med Res 1988; 87:434-439.

Rajagopalan PK, Das PK, Subramanian S, Vanamail P, Ramaiah KD. Bancroftian filariasis in Pondicherry, south India: 1. Pre-control epidemiological observations. Epidemiol Infect 1989; 103(3):685-692.

Rajagopalan PK, Kazmi SJ, Mani TR. Some aspects of transmission of Wuchereria bancrofti and ecology of the vector Culex pipiens fatigans in Pondicherry. Indian J Med Res 1977; 66(2):200-215.

Rajagopalan PK, Panicker KN, Pani SP. Impact of 50 years of vector control on the prevalence of Brugia malayi in Shertallai area of Kerala state. Indian J Med Res 1989;89:418-425.

Rajagopalan PK, Shetty PS, Arunachalam N. A filariasis survey in Pondicherry villages. Indian J Med Res 1981; 73 Suppl:73-77.

Rajasekariah GR, Parab PB, Chandrashekar R, Deshpande L, Subrahmanyam D. Pattern of Wuchereria bancrofti microfilaremia in young and adolescent school children in Bassein, India, an endemic area for lymphatic filariasis. Ann Trop Med Parasitol 1991; 85(6):663-665.

Rajendran G, Krishnamoorthy K, Sabesan S, Panicker KN. Attachment behaviour of immatures of Mansonia annulifera (Theobald), 1901. Indian J Med Res 1989; 89:192-197.

Rajendran G, Panicker KN, Krishnamoorthy K, Sabesan S, Snehlatha, Radhakrishnan R. Current status of filariasis in Chavakad taluk, Trichur district, Kerala. J Commun Dis

1997; 29(4):333-343.

Ramaiah KD, Das PK, Appavoo NC, Ramu K, Augustin DJ, Kumar KN et al. A programme to eliminate lymphatic filariasis in Tamil Nadu state, India: compliance with annual single-dose DEC mass treatment and some related operational aspects. Trop Med Int Health 2000; 5(12):842-847.

Ramaiah KD, Das PK, Arunachalam N, Rajavel AR, Paily KP. Observations on population density of Culex quinquefasciatus and transmission indices of Bancroftian filariasis during and after Integrated Vector Management strategy. J Commun Dis 1992; 24(3): 173-184.

Ramaiah KD, Das PK, Dhanda V. Estimation of permissible levels of transmission of bancroftian filariasis based on some entomological and parasitological results of a -5-year vector control programme. Acta Trop 1994; 56(l):89-96.

Ramaiah KD, Das PK, Michael E, Guyatt H. The economic burden of lymphatic filariasis in India. Parasitol Today 2000; 16(6):251-253.

Ramaiah KD, Das PK. Non-involvement of nulliparous females in the transmission of bancroftian filariasis. Acta Trop 1992; 52(2-3): 149-153.

Ramaiah KD, Das PK. Seasonality of adult Culex quinquefasciatus and transmission of bancroftian filariasis in Pondicherry, south India. Acta Trop 1992; 50(4):275-283.

Ramaiah KD, Guyatt H, Ramu K, Vanamail P, Pani SP, Das PK. Treatment costs and loss of work time to individuals with chronic lymphatic filariasis in rural communities in south India. Trop Med Int Health 1999; 4(1): 19-25.

Ramaiah KD, Kumar KN, Ramu K, Pani SP, Das PK. Functional impairment caused by lymphatic filariasis in rural areas of south India. Trop Med Int Health 1997;2(9):832-838.

Ramaiah KD, Kumar KN, Ramu K. Knowledge and beliefs about transmission, prevention and control of lymphatic filariasis in rural areas of south India. Trop Med Int Health 1996; l(4):433-438.

Ramaiah KD, Pani SP, Balakrishnan N, Sadanandane C, Das LK, Mariappan T et al. Prevalence of bancroftian filariasis & its control by single course of diethyl carbamazine in a rural area in Tamil Nadu. Indian J Med Res 1989; 89:184-191.

Ramaiah KD, Radhamani MP, John KR, Evans DB, Guyatt H, Joseph A et al. The impact of lymphatic filariasis on labour inputs in southern India: results of a multi-site study. Ann Trop Med Parasitol 2000; 94(4):353-364.

Ramaiah KD, Ramu K, Guyatt H, Kumar KN, Pani SP. Direct and indirect costs of the acute form of lymphatic filariasis to households in rural areas of Tamil Nadu, south India. Trop Med Int Health 1998; 3(2): 108-115.

Ramaiah KD, Ramu K, Kumar KN, Guyatt H. Epidemiology of acute filarial episodes caused by Wuchereria bancrofti infection in two rural villages in Tamil, Nadu, south India. Trans R Soc Trop Med Hyg 1996; 90(6):639-643.

Ramaiah KD, Vijay Kumar KN. Effect of lymphatic filariasis on school children. Acta Trop 2000; 76(2): 197-199.

Ramaiah KD. A rational approach to the control of filariasis in India. Natl Med J India 1993; 6(3): 114-116.

Ramu K, Ramaiah KD, Guyatt H, Evans D. Impact of lymphatic filariasis on the productivity of male weavers in a south Indian village. Trans R Soc Trop Med Hyg 1996; 90(6):669-670.

Ramzy RM, Gad AM, Faris R, Weil GJ. Evaluation of a monoclonal-antibody based antigen assay for diagnosis of Wuchereria bancrofti infection in Egypt. Am J Trop Med Hyg 1991; 44(6):691-695.

Rangabashyam N, Gnanaprakasam D, Krishnaraj B, Manohar V, Vijayalakshmi SR. Spectrum of benign breast lesions in Madras. J R Coll Surg Edinb 1983; 28(6):369-373.

Rao BV, Ramakrishna RP, Subba RK, Patnaik B. On the occurrence of Stephanofilaria assamensis var: bubalensis in Cuddapah district: Andhra Pradesh. Indian Vet J 1979; 56(3):250-251.

Rao CK Filariasis control in Primary Health Care. J Commun Dis 1982; 14(3):206-211.

Rao CK Indices for evaluation of filariasis control in India-a critique. J Commun Dis 1982; 14(1):9-15.

Rao CK Prasad KR, Nath VV, Narasimham MV, Sundaram RM, Dutta KK et al. Prevalence and incidence rates of microfilaraemia and filaria disease in the East Godavari district of Andhra Pradesh. Indian J Med Res 1982; 75:23-27.

Rao CK, Chandrasekharan A, Cherian C. Frequency and duration of acute filarial attacks in persons in Brugia malayi endemic community. Indian J Med Res 1982; 75:813-815.

Rao CK, Chandrasekharan A, Kaul SM, Narasimham MV, Sharma SP. Relative effectiveness of different methods of control of Brugian filariasis in India. Indian J Med Res 1980; 72:194-202.

Rao CK, Datta KK, Sundaram RM, Ramaprasad K, Rao JS, Venkatanarayana M et al. Epidemiological studies on bancroftian filariasis in East Godavari district (Andhra Pradesh): baseline filariometric indices. Indian J Med Res 1980; 71:712-720.

Rao CK, Kumar S. Role of filariasis in endomyocardial fibrosis. J Commun Dis 1982; 14(2):91-95.

Rao CK, Narasiraham MV, Krishna RC. Observations on duration of microfilaraeraia in rural bancroftian filariasis. .1 Commun Dis 1982; 14(2): 138-140.

Rao CK, Prasad KR, Nath W, Narasimham MV, Rao CK, Venkatanarayana M et al. Impact of control measures on prevalence and incidence of bancroftian filariasis in East Godavari district (Andhra Pradesh). Indian J Med Res 1982; 75:515-520.

Rao CK, Ramaprasad K, Narasimham MV, Rao CK, Jaggi OP. Epidemiology of bancroftian filariasis in East Godavari district (Andhra Pradesh)--incidence of tropical pulmonary eosinophilia. Indian J Med Res 1981; 74:517-523.

Rao CK, Rao PK, Prasad KR, Rao CK. Epidemiological studies on bancroftian filariasis in East Godavari district (Andhra Pradesh): II. Periodicity of microfilaria. J Commun Dis 1980; 12(3): 164-166.

Rao CK, Sharma SP. Control of filariasis in India. J Commun Dis 1986; 18(4):276-282.

Rao CK, Sundaram RM, Venkatanarayana M, Rao JS, Chandrasekharan A, Rao CK. Epidemiological studies on bancroftian filariasis in East Godavari District (Andhra Pradesh): entomological aspects. J Commun Dis 1981; 13(2):81-91.

Rao CK. Experience in using diethylcarbamazine (DEC) in the control of lymphatic filariasis in India. J Commun Dis 1985; 17(l):36-45.

Rao CK. On the national filaria control programme. Indian J Public Health 1979; 23(1)13-6.

Rao NS, Murthy NS, Marwah SM. A statistical analysis of age factor amongst filariasis carriers in areas with different infection rates. Indian J Med Res 1973; 61(6):943-949.

Rao PK, Venkatanarayana M, Narasimham MV, Rao CK. Effect of diethylcarbamazine medicated common salt on Wuchereria bancrofti prophylaxis. J Commun Dis 1980; 12(4):205-209.

Rath RN, Das RK, Mishra G, Mohapatra BN, Ramakrishna C. Bancroftian filariasis in two selected rural communities in Puri district: Orissa-a comparative study of filariometric data. J Commun Dis 1984; 16(2): 104-112.

Rath RN, Mohapatra BN, Das B. Detection of a new focus of Brugia malayi infection in Orissa. J Commun Dis 1989; 21(l):39-40.

Ravindran B, Sahoo PK, Dash AP. Lymphatic filariasis and malaria: concomitant parasitism in Orissa, India. Trans R Soc Trop Med Hyg 1998; 92(l):21-23.

Ravindran B, Satapathy AK, Sahoo PK, Babu Geddam JJ. Protective immunity in human Bancroftian filariasis: inverse relationship between antibodies to micro filarial sheath and circulating filarial antigens. Parasite Immunol 2000; 22(12):633-637.

Ravindranathan TC, Roychowdhury SP, Russel S, Rao CK. Filaricidal effect of primaquine. J Commun Dis 1982; 14(l):88-89.

Ravindranathan TC, Sethumadhavan KV, Roychowdhury SP, Babu CS, Kanoujia KH, Narasimham MV et al. Seasonal variation in microfilaraemia (bancrofti) density and filaria disease. J Commun Dis 1980; 12(2):73-77.

Ray D, Abel R. Hypereosinophilia in association with pulmonary tuberculosis in a rural population in south India. Indian J Med Res 1994; 100:219-222.

Reddy GS, Venkateswaralu N. Mass administration of DEC-medicated salt for filariasis control in the endemic population of Karaikal, south India: implementation and impact assessment. Bull World Health Organ 1996; 74(1): 85-90.

Reddy MV, Malhotra A, Harinath BC. Detection of circulating antigen in bancroftian filariasis by sandwich ELISA using filarial serum IgG. J Helminthol 1984; 58(3):259-262.

Reuben R, Rajendran R, Sunish IP, Mani TR, Tewari SC, Hiriyan J et al. Annual single-dose diethylcarbamazine plus ivermectin for control of bancroftian filariasis: comparative efficacy with and without vector control. Ann Trop Med Parasitol 2001; 95(4):361-378.

Richard SA, Mathieu E, Addiss D, Sodahlon YK: A survey of treatment practices and burden of lymphoedema in Togo. Trans Roy Soc Trop Med Hyg. 2007, 101 (4): 391-397. 10.1016/j.trstmh.2006.08.011.

Rochet MJ. A simple deterministic model for bancroftian filariasis transmission dynamics. Trop Med Parasitol 1990; 41(3):225-233.

Rozeboom LE, Bhattacharya NC, Gilotra SK. Observations on the transmission of filariasis in urban Calcutta. Am J Epidemiol 1968; 87(3):616-631.

Rudra SK, Chandra G. Comparative epidemiological studies on lymphatic filariasis, between tribal and non-tribal populations of Bankura district, West Bengal, India. Ann Trop Med Parasitol 2000; 94(4):365-372.

Russel S, Rao AR, Khamre JM, Praharaj BC, Rao CK. Disappearance of some Brugia malayi foci from Madhya Pradesh, Orissa and Tamil Nadu and coexistence of Malayan and Bancroftian filariasis in some pockets. J Commun Dis 1980; 12(4):183-187.

Russel S, Rao CK, Mehta PK, Roy NK. Filariasis in Dadra and Nagar Haveli. J Commun Dis 1982; 14(2): 157-160.

Russel S, Rao CK, Rao CK. Prevalence of nonfilarial elephantiasis in selected towns in India. J Commun Dis 1983; 15(3):216-218.

Ryan T: A search for consensus on the staging of lymphedema. *Lymphology* 2004, 37:180-181

Sabesan S, Krishnamoorthy K, Panicker KN, Vanamail P. The dynamics of micro filar aemia and its relation with development of disease in periodic Brugia malayi infection in south India. Epidemiol Infect 1991; 107(2):453-463.

Sabesan S, Kumar NP, Krishnamoorthy K, Panicker KN. Seasonal abundance & biting behaviour of Mansonia annulifera, M. uniformis & M. indiana & their relative role in the transmission of malayan filariasis in Shertallai (Kerala state). Indian J Med Res 1991; 93:253-258.

Sabesan S, Palaniyandi M, Das PK, Michael E. Mapping of lymphatic filariasis in India. Ann Trop Med Parasitol 2000;94:591-606.

Sabesan S, Palaniyandi M, Das PK, Michael E. Mapping of lymphatic filariasis in India. Ann Trop Med Parasitol 2000; 94(6):591-606.

Sabesan S. Albendazole for mass drug administration to eliminate lymphatic filariasis. Lancet Infect Dis 2006;6:684-5.

Sahare KN, Anandharaman V, Meshram VG, Meshram SU, Gajalakshmi D,Goswami K, et al. In vitro effect of four herbal plants on the motility of Brugia malayi microfilariae. Indian J Med Res 2008; 127: 467-471.

Sahoo PK, Geddam JJ, Satapathy AK, Mohanty MC, Ravindran B. Bancroftian filariasis: prevalence of antigenaemia and endemic normals in Orissa, India. Trans R Soc Trop Med Hyg 2000; 94(5):515-517.

Sahu KC, Jena DC. Studies on the use of strontium bromide (Ekzebrol) in eczematoid conditions of fdariasis and reaction of leprosy. Indian Pract 1967; 20(1):121-128.

SaintAndreA,et al.2002.The role of endosymbioticWolbachia bacteria in the pathogenesis of river blindness. Science 295:1892–1895.

Sangha HK, Chitkara NL. Icidence of filariasis in the industrial population of amritsar. Indian J Pathol Bacteriol 1975; 18(l):41-45.

Sanjeevi CB, Narayanan PR. Antifilarial and anti PPD IgM antibodies in cord blood. Indian J Pediatr 1989; 56(2):207-211.

Sant MV, Gatlewar WN, Menon TU. Epidemiological and biochemical studies in filariasis in four villages near Bombay. Prog Drug Res 1974; 18:269-275.

Santhanam S, Kumar H, Sethumadhavan KV, Chandrasckharan A, Jain DC, Malhotra A et al. Detection of Wuchereria bancrofti antigen in scrum and finger prick blood samples by enzyme immunoassay: field evaluation. Trop Med Parasitol 1989; 40(4):440-444.

Sarma RV, Vallishayee RS, Rao RS, Prabhakar R, Tripathy SP. Use of mebendazole in combination with DEC in bancroftian filariasis. Indian J Med Res 1988; 87:579-583.

Sarma RV, VaUishayee RS, Mayurnath S, Narayanan PR, Radhamani MP, Tripathy SP. Prevalence survey of filariasis in two villages in Chingleput district of Tamil Nadu. Indian J Med Res 1987; 85:522-530.

Satapathy AK, Sartono E, Sahoo PK, Dentener MA, Michael E, Yazdanbakhsh M, Ravindran B. Human bancroftian filariasis: immunological markers of morbidity and infection. Microbes Infect. 2006; 8:2414–2423. [PubMed: 16839794]

Saxena H, Singh SN, Samuel KC, Ajwani KD. Nodular breast lesions caused by filarial worms: report of three cases. Am J Trop Med Hyg 1975; 24(5):894-896.

Saxena VK. Biometrical variations in haematophagous arthropods. II. DV/D ratio of male terrninalia of wild Culex quinquefasciatus SAY (Diptera: Culicidae) in India. J Commun Dis 1990; 22(l):60-66.

Schmitz KH, Ahmed RL, Troxel A, Cheville A, Smith R, Lewis-Grant L, et al. Weight lifting in women with breast-cancer-related lymphedema. *N Engl J Med*. 2009 Aug 13. 361(7):664-73.

Semnani RT, Nutman TB. 2004. Toward an understanding of the interaction between filarial parasites and host antigen-presenting cells. Immunol. Rev. 201:127–138.

Sethumadhavan KV, Babu CS, Roychowdhury SP, Russel S. Effect of high dosage of diethylcarbamazine on microfilariae after prolonged exposure to low dosage. J Commun Dis 1982; 14(l):84-85.

Sharma GK, Rao CK, Sharma SP, Sundaram RM, Ghosh TK, Raina VK et at. Relative impact of integrated vector control strategy vis-a-vis conventional control strategy on bancroftian filariasis in Pondicherry. J Commun Dis 1986; 18(4):267-275.

Sharma RS, Raina VK, Rao CK. A filariasis survey among the migratory labourers at Alwar, Rajasthan. J Commun Dis 1986; 18(4):287-290.

Sharma S, Sharma M, Rathaur S. Bancroftian filariasis in the Varanasi region of north India: an epidemiological study. Ann Trop Med Parasitol 1999; 93(4):379-387.

Sharma SN, Sharma T, Prasad H. Impact of Spherix (Bacillus sphaericus B-101, serotype H5a, 5b) spraying on the control of mosquito breeding in rural areas of Farrukhabad District, Uttar Pradesh. Indian J Malariol 1998; 35(4):185-196.

Sharma SP, Biswas H, Das M, Dwivedi SR. Present status of the filariasis problem in India. J Commun Dis 1983; 15(l):53-60.

Sharma SP, Biswas H, Das M, Kosare GJ, Sharma GK. Control of Bancroftian filariasis with diethylcarbamazine in rural areas of Valsad district, Gujarat State. J Commun Dis 1984; 16(4):282-286.

Sharma SP, Biswas H, Pandey DS, Pandey RS, Das M, Sharma GK. Control of bancroftian filariasis in a rural area through selected treatment with diethylcarbamazine. J Commun Dis 1987; 19(4):322-325.

Sharma SP, Kosare GJ, Biswas H, Das M, Trivedi GK, Sharma GK. Control of bancroftian filariasis in rural areas through selected treatment with diethylcarbamazine. J Commun Dis 1986; 18(4):283-286.

Shenoy RK, Dalia S, John A, Suma TK, Kumaraswami V. Treatment of the microfilaraemia of asymptomatic brugian filariasis with single doses of ivermectin, diethylcarbamazine or albendazole, in various combinations. Ann Trop Med Parasitol 1999;93:643

Shenoy RK, John A, Babu BS, Suma TK, Kumaraswami V. Two-year follow-up of the microfilaraemia of asymptomatic brugian filariasis, after treatment with two, annual, single doses of ivermectin, diethylcarbamazine and albendazole, in various combinations. Ann Trop Med Parasitol 2000; 94(6):607-614.

Shenoy RK, Kumaraswami V, Rajan K, Thankom S, Jalajakumari. A comparative study of the efficacy and tolerability of single and split doses of ivermectin and diethylcarbamazine in periodic brugian filariasis. Ann Trop Med Parasitol 1993; 87(5):459-467.

Shenoy RK, Kumaraswami V, Rajan K, Thankom S, Jalajakumari. Ivermectin for the treatment of periodic malayan filariasis: a study of efficacy and side effects following a single oral dose and retreatment at six months. Ann Trop Med Parasitol 1992; 86(3):271-278.

Shenoy RK, Kumaraswami V, Suma TK, Rajan K, Radhakuttyamma G. A double-blind, placebo- controlled study of the efficacy of oral penicillin, diethylcarbamazine or local treatment of the affected limb in preventing acute adenolymphangitis in lymphoedema caused by brugian filariasis. Ann Trop Med Parasitol. 1999; 93:367–377. [PubMed: 10656038].

Shenoy RK, Sandhya K, Suma TK, Kumaraswami V. A preliminary study of filariasis related acute adenolymphangitls with special reference to precipitating factors and treatment modalities. Southeast Asian J Trop Med Public Health 1995; 26(2):301-305.

Shenoy RK, Suma TK, John A, Arun SR, Kumaraswami V, Fleckenstein LL, et al. The pharmacokinetics, safety and tolerability of the co-administration of diethylcarbamazine and albendazole. Ann Trop Med Parasitol 2002;96:603-14.

Sunish IP, Rajendran R, Mani TR, Dash AP, Tyagi BK. Evidence for the use of albendazole for the elimination of lymphatic filariasis. Lancet Infect Dis 2006;6:125-6.

Shenoy RK, Suma TK, Rajan K, Kumaraswami V: Prevention of acute adenolymphangitis in brugian filariasis: Comparison of the efficacy of ivermectin and diethylcarbamazine, each combined with local treatment of the affected limb. Ann Trop Med Parasitol. 1998, 92 (5): 587-594. 10.1080/00034989859285.

Shoho C. [Setaria species from water buffalo of Southeast Asia and from Caffer Buffalo of Africa: comparative study with Setaria spp. from cattle of the respective areas (author's transl)]. Ann Parasitol Hum Comp 1976; 51(5):577-588.

Shriram AN, Sugunan AP, Murhekar MV, Sehgal SC. Little Andaman Island, a new locus of infection with noclurnally periodic Wuchereria bancrofti. Indian J Med Res 1996; 104:166-170.

Simonsen PE. Immune reactions to Wuchereria bancrofti infections in Tanzania. II.

Characterization of the serum-mediated adherence reaction of leucocytes to microfilariae in vitro. Trans R Soc Trop Med Hyg 1983; 77(6):834-839.

Singaravelu G, Mahalingam S, Sumathy S. Estimation of different degrees of provocation by DEC (diethyl carbamazine citrate) medication in bancroftian filariasis in Vellore, Tamilnadu. Indian J Exp Biol 1999; 37(11):1142-1143.

Singh D. The Culex pipiens fatigans problem in South-East Asia with special reference to urbanization. Bull World Health Organ 1967; 37(2):239-243.

Singh S, Bora D, Sharma RC. A study of filarial transmission in a non-endemic area of Pathankot (Punjab). J Commun Dis 2000; 32(l):61-64.

Singhvi A, Pulimood RB, John TJ, Babu PG, Samuel BU, Padankatti T et al. The prevalence of markers for hepatitis B and human immunodeficiency viruses, malarial parasites and microfilaria in blood donors in a large hospital in south India. J Trop Med Hyg 1990; 93(3):178-182.

Sivam NS, Jayanthi S, Ananthakrishnan N, Elango A, Yuvaraj J, Hoti SL et al. Tropical vaginal hydroceles: are they all filarial in origin? Southeast Asian J Trop Med Public Health 1995; 26(4):739-742.

Smith CE, Glanville VJ. A school for the study of tropical medicine. J Trop Med Hyg 1973; 76(8): 194-197.

Smith DH, Wilson T, Berezancev JA, Lykov V, Paing M, Chari MV et al Evaluation of the Dirofilaria immitis filarial skin test antigen in the diagnosis of filariasis. Bull World Health Organ 1971; 44(6):771-782.

Smith EK, Tunks E, Tuttle RJ. Filarial chyluria: a case discovered in Ontario. Can Med Assoc J 1971; 105(12):1315-1317.

Smith RJ, Cotter TP, Williams JF, Guderian RH. Vascular perfusion of Onchocerca volvulus nodules. Trop Med Parasitol 1988; 39 Suppl 4:418-421.

Southeast Asian J Trop Med Public Health 1986; 17: 457-64.

Southgate BA, Bryan JH. Factors affecting transmission of Wuchereria bancrofti by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. Trans R Soc Trop Med Hyg 1992; 86(5):523-530.

Spencer I. Surgical aspects of filariasis. Trop Doct 1974; 4(l):26-30.

Srivastava RN, Bhushan V, Prasad BG. Quantitative concept in the assessment of filariasis: observations in villages of the Rural Health Training Centre, Sarojini Nagar, Lucknow. Indian J Med Res 1969; 57(10):1975-1981.

Srivastava RN, Prasad BG. An epidemiological study of filariasis in the villages of the Rural Health Training Centre, Sarojini Nagar, Lucknow. Indian J Med Res 1969; 57(3):528-542.

Srividya A, Das PK, Subramanian S, Ramaiah KD, Grenfell BT, Michael E et al. Past exposure and the dynamics of lymphatic filariasis infection in young children. Epidemiol Infect 1996; 117(1):195-201.

Srividya A, Das PK. Utility of the force of infection model for assessing changes in the dynamics of bancroftian filarial infections. Southeast Asian J Trop Med Public Health 1994; 25(1):201-207.

Srividya A, Krishnamoorthy K, Sabesan S, Panicker KN, Grenfell BT, Bundy DA. Frequency distribution of Brugia malayi microfilariae in human populations. Parasitology 1991; 102 Pt 2:207-212.

Srividya A, Lall R, Ramaiah KD, Ramu K, Hoti SL, Pani SP et al. Development of rapid assessment procedures for the delimitation of lymphatic filariasis-endemic areas. Trop Med Int Health 2000; 5(1):64-71.

Srividya A, Pani SP, Rajagopalan PK, Bundy DA, Grenfell BT. The dynamics of infection and disease in bancroftian filariasis. Trans R Soc Trop Med Hyg 1991; 85(2):255-259.

Srividya A, Pani SP. Filariasis and blood groups. Natl Med J India 1993; 6(5):207-209.

Stingl P, Ross M, Gibson DW *et al.* A diagnostic 'patch test' for onchocerciasis using topical diethylcarbamazine. *Trans. R. Soc. Trop. Med. Hyg.* 78(2), 254–258 (1984).

Stuiver PC, Wismans PJ, Schornagel R. [Tropical eosinophils as an imported disease in The Netherlands]. Ned Tijdschr Geneeskd 1991; 135(7):283-286.

Sturchler D, Imbach P, Gartmann J, Degremont A. [Clinical aspects, diagnosis and therapy of tropical pulmonary eosinophilia]. Schweiz Med Wochenschr 1978; 108(38): 1461-1464.

Subrahmanyam M. Observations on bancroftian filariasis in Wardha, Central India. J Assoc Physicians India 1981; 29(9):721-724.

Subramanian S, Krishnamoorthy K, Ramaiah KD, Habbema .ID, Das PK, Plaisier AP. The relationship between microfilarial load in the human host and uptake and development of Wuchereria bancrofti microfilariae by Culex quinquefasciatus: a study under natural conditions. Parasitology 1998; 116 (Pt 3):243-255.

Subramanian S, Pani SP, Das PK, Rajagopalan PK. Bancroftian filariasis in Pondicherry, south India: 2. Epidemiological evaluation of the effect of vector control. Epidemiol Infect 1989; 103(3):693-702.

Subramanian SP. Microfdaria in anterior chamber of the eye. J Indian Med Assoc 1966;47(10):503-504.

Subramanyam RG, Vengatesvarlou N, Das PK, Vanamail P, Vijayan AP, Kala S et al. Tolerability and efficacy of single-dose diethyl carbamazine (DEC) or ivermectin in the clearance of Wuchereria bancrofti microfilaraemia in Pondicherry, south India. Trop Med Int Health 2000; 5(ll):779-785.

Subramanyam VR. Filariasis and malaria sera from Orissa lack HTLV-III antibody reactivity. J Commun Dis 1989; 21(4):282-284.

Sunny PJ, Jain DC, Ghosh TK. Study on the role of human behaviour on environmental modification leading to mansonioides control. J Commun Dis 1988; 20(2): 127-130.

Surendran K, Pani SP, Soudarssananc MB, Srinivasa DK, Bordolai PC, Subramanian S. Natural history, trend of prevalence and spectrum of manifestations of Bancroftian filarial disease in Pondicherry (South India). Acta Trop 1996; 61(1):9-18.

T.F. Schäberle et al.Corallopyronin A – A promising antibiotic for treatment of filariasis International Journal of Medical Microbiology 304 (2014) 72–78

Takeda K, Kaisho T, Akira S. 2003. Toll-like receptors. Annu. Rev.Immunol. 21:335–376.

Tatochenko VK. [Control of fdariasis in India]. Med Parazitol (Mosk) 1967: 36(5):613-617.

Tatochenko VK. [Some aspects of the medico-geographic study of India]. Sov Zdravookhr 1968; 27(3):76-80.

Tewari SC, Hiriyan J, Reuben R. Epidemiology of subperiodic Wuchereria bancrofti infection in the Nicobar Islands, India. Trans R Soc Trop Med Hyg 1995; 89(2):163-166.

Theodore JG, Kaliraj P, Jayachandran S, Jayaraman K. Cloning, over-expression and evaluation of a recombinant fusion protein of Wuchereria bancrofti towards its application as a diagnostic agent for bancroftian filariasis. Parasitology 1993; 106 (Pt4):413-420.

Thommandru J, Higashi GI, Santhanam S, Malhotra A. Quantitative cellular cytotoxicity to Brugia malayi microfilariae: in vitro studies of sera from an area endemic for filariasis in Andhra Pradesh, India. J Egypt Soc Parasitol 1991; 21(1):1-13.

Turner JD, et al. 2009. Wolbachia lipoprotein stimulates innate and adaptive immunity through Toll-like receptors 2 and 6 to induce disease manifestations of filariasis. J. Biol. Chem. 284:22364 –22378.

van der Walt JC, Perks TJ, Zeeman BJ, Bruce-Chwatt AJ, Graewe FR. Modified Charles procedure using negative pressure dressings for primary lymphedema: a functional assessment. *Ann Plast Surg*. 2009 Jun. 62(6):669-75.

Vanamail P, Ramaiah KD, Das PK. Risk of infection of Wuchereria bancrofti to humans by Culex quinquefasciatus in Pondicherry and its relationship with microfilaria prevalence. Acta Trop 1993; 55(4):237-247.

Vanamail P, Ramaiah KD. Biting periodicity index of Culex quinquefasciatus & its relationship with microfilaria periodicity in Pondicherry. Indian J Med Res 1991;93:379-383.

Vanamail P, Subramanian S, Das PK, Pani SP, Rajagopalan PK, Bundy DA et al. Estimation of age-specific rates of acquisition and loss of Wuchereria bancrofti infection. Trans R Soc Trop Med Hyg 1989; 83(5):689-693.

Vanamail P, Subramanian S, Das PK, Pani SP, Rajagopalan PK. Estimation of fecundic life span of Wuchereria bancrofti from longitudinal study of human infection in an endemic area of Pondicherry (south India). Indian J Med Res 1990; 91:293-297.

Vanamail P, Subramanian S, Rajagopalan PK. A mathematical analysis of various factors involved in transmission of bancroftian filariasis in Pondicherry. Indian J Med Res 1990;91:289-292.

Varghese R, Raghuveer CV, Pai MR, Bansal R. Microfilariae in cytologic smears: a report of six cases. Acta Cytol 1996; 40(2):299-301.

Varma AK, Sahai BN, Singh SP, Lakra P, Shrivastava VK. On Setaria digitata, its specific characters, incidence and development in Aedes vittatus and Armigeres obturbans in India with a note on its ectopic occurrence. Z Parasitenkd 1971; 36(l):62-72.

Varma BK, Kapcar A, Maingi NS. Filariasis in a 12-year-old girL Am J Dis Child 1979; 133(1):100.

Varma RN, Boparai MS. The health and medical care of troops and their families. Indian J

Public Health 1972; 16(3): 136-140.

Varma RN. Filariasis as a health problem in the armed forces and its controL Indian J Med Sci 1974; 28(2):65-70.

Vijayalakshmi N: Role of aerobic bacteria in the aetiopathogenesis of acute adenolymphangitis in bancroftian filarial lymphoedema. PhD. 1997, Pondicherry, India: Jawaharlal Institute of Post-Graduate Medical Education and Research

Vijayan VA, Ningegowda N. Susceptibility difference in two populations of Culex quinquefasciatus Say (Diptera: Culicidae) to three synthetic pyrethroids. Southeast Asian J Trop Med Public Health 1993; 24(3):540-543.

Vijayan VK. Tropical pulmonary eosinophilia. Indian J Chest Dis Allied Sci 1996; 38(3):169-180.

von Lichtenberg F. The Wellcome Trust lecture. Inflammatory responses to filarial connective tissue parasites. Parasitology. 1987; 94(Suppl):S101–S122. [PubMed: 3295688]

Wabo Pone J, Bilong Bilong CF, Mpoame M. In vitro nematicidal activity of extracts of Canthium mannii (Rubiaceae), on different life-cycle stages of Heligmosomoides polygyrus (Nematoda, Heligmosomatidae). J Helminthol 2009; 1-10.

Walker, M., Little, M.P., Wagner, K.S., Soumbey-Alley, E.W., Boatin, B.A., Basanez, M.G., 2012. Density-dependent mortality of the human host in onchocerciasis: relationships between microfilarial load and excess mortality. PLoS Negl. Trop. Dis. 6, e1578

Warren AG, Brorson H, Borud LJ, Slavin SA. Lymphedema: a comprehensive review. *Ann Plast Surg*. 2007 Oct. 59(4):464-72. .

Weil G, Lammie PJ, Weiss N. The ICT filariasis test: A rapid format antigen test for diagnosis of bancrofitian flariasis. Parasitol Today 1997; 13: 401-404.

Weil GJ, Hussain R, Kumaraswami V, Tripathy SP, Phillips KS, Ottesen EA. Prenatal allergic sensitization to helminth antigens in offspring of parasite-infected mothers. J Clin Invest 1983; 71(5): 1124-1129.

Weil GJ, Jain DC, Santhanam S, Malhotra A, Kumar H, Sethumadhavan KV et al. A monoclonal antibody-based enzyme immunoassay for detecting parasite antigenemia in bancroftian filariasis. J Infect Dis 1987; 156(2):350-355.

Weil GJ, Kumar H, Santhanam S, Sethumadhavan KV, Jain DC. Detection of circulating parasite antigen in bancroftian filariasis by counterimmunoelectrophoresis. Am J Trop Med Hyg 1986; 35(3):565-570.

Weil GJ. Re: S.P. Pani et al. Evaluation of the ICT whole blood antigen card test to detect infection due to nocturnally periodic Wucheria bancrofti in South India. Tropical Medicine and International Health 5, 359-363. Trop Med Int Health 2000; 5(11):832-833.

WHO, 2011. Metrics Disability-Adjusted Life Year (DALY). Health statis-tics and health information systems,

http://www.who.int/healthinfo/global burden disease/metrics daly/en/ WHO;1992.

World Health Organization (2010) WHO global programme to eliminate lymphatic filariasis progress report for 2000–2009 and strategic plan 2010–2020. Geneva: World Health Organization.

Wu X, Liu N. 2010. The role of Ang/Tie signaling in lymphangiogenesis.

www.thelancet.com/infection India tackles lymphatic filariasis Sanjeet Bagcchi reports Vol 15 April 2015

Yadav RS, Sharma VP, Upadhyay AK. Field trial of Bacillus sphaericus strain B-101 (serotype H5a, 5b) against filariasis and Japanese encephalitis vectors in India. J Am Mosq Control Assoc 1997; 13(2):158-163.

Yadava RL, Biswas H, Rao CK, Narasimham MV, Thapar BR. Success of integrated methods of control of mosquitoes in India-a review. J Commun Dis 1990; 22(2):79-85.

Yesudian P, Kumaraswami V. Filariasis. The scourge of India. Int J Dermatol 1989; 28(3): 192-194.

Zhang S, Li B, Weil GJ. Human antibody responses to Brugia malayi antigens in brugian filariasis. Int J Parasitol 1999; 29(3):429-436.

Annexure-1

Helminth Coinfection Results

ANNEXURE 1

TABLE 1
Co-infection with helminths

Village	ICT+ Stool+	ICT+ Stool-	ICT-Stool+	ICT- Stool-	Total
Vellathukottai	123	72	736	563	1494
Mambakkam	3	15	120	857	995
Devendavakkam	2	60	122	871	1055
Kanchipadi	33	112	65	326	537
Total	161	259	1043	2617	4081

ICT + = Positive for filarial antigen
Stool + = Positive for helminth

TABLE 2

DISTRIBUTIONS OF BCG SCAR, INFECTIONS AND PPD REACTION BY GENDER

	Gender				Total	
	F		M			
	N	%	N	%	N	%
Total	2753	100.0	2343	100.0	5096	100.0
BCG Scar						
Absent	20X5	75.7	1627	69.4	3712	72.8
Present	668	24.3	716	30.6	1384	27.2
Stool Helminth Infection						
Negative	1835	66.7	1630	69.6	3465	68.0
Positive	918	33.3	713	30.4	1631	32.0
Circulating Filarial Ag						
Negative	2543	92.4	2088	89.1	4631	90.9
Positive	210	7.6	255	10.9	465	9.1
Stool or Circulating Ag						
Negative	1707	62.0	1466	62.6	3173	62.3
Positive	1046	38.0	8,77	37.4	1923	37.7
PPD Reaction > 10						
Missing	326	11.8	307	13.1	633	12.4
No	1108	40.2	819	35.0	1927	37.8
Yes	1319	47.9	1217	51.9	2536	49.8
PPD Reaction > 12						
Missing	326	11.8	307	13.1	633	12.4
No	1275	46.3	969	41.4	2244	44.0
Yes	1152	41.8	1067	45.5	2219	43.5

TABLE 3

DISTRIBUTIONS OF BCG SCAR, INFECTIONS AND PPD REACTION BY AGE QUARTILES

	Age Quartiles								Total	
	<=14		(14,28)		(28,42)		>42			
	N	%	N	%	N	%	N	%	N	%
Total	1324	100	1299	100	1279	100	1194	100	5096	100
BCG Scar										
Absent	935	70.6	1186	91.3	813	63.6	778	65.2	3712	72.8
Present	389	29.4	113	8.7	466	36.4	416	34.8	1384	27.2
Stool Helminth Infection										
Negative	931	70.3	896	69.0	869	67.9	769	64.4	3465	68.0
Positive	393	29.7	403	31.0	410	32.1	425	35.6	1631	32.0
Circulating Filarial Ag										
Negative	1231	93.0	1160	89.3	1148	89.8	1092	91.5	4631	90.9
Positive	93	7.0	139	10.7	131	10.2	102	8.5	465	9.1
Stool or Circulating Ag										
Negative	867	65.5	807	62.1	788	61.6	711	59.5	3173	62.3
Positive	457	34.5	492	37.9	491	38.4	483	40.5	1923	37.7
PD Reaction > 10										
Missing	75	5.7	260	20.0	156	12.2	142	11.9	633	12.4
No	1017	76.8	443	34.1	247	19.3	220	18.4	19.27	37.8
Yes	232	17.5	596	45.9	876	68.5	832	69.7	2536	49.8
PPD Reaction > 12										
Missing	75	5.7	260	20.0	156	12.2	142	11.9	633	12.4
No	1068	80.7	557	42.9	327	25.6	292	24.5	2244	44.0
Yes	181	13.7	482	37.1	796	62.2	760	63.7	2219	43.5

TABLE 4

DISTRIBUTION OF HELMINTH INFECTIONS BY BCG SCAR STATUS

	BCG Scar				Total	
	Absent		Present			
	N	%	N	%	N	%
Total	3712	100.0	1384	100.0	5096	100.0
Stool Helminth Infection						
Negative	2484	66.9	981	70.9	3465	68.0
Positive	1228	33.1	403	29.1	1631	32.0
Circulating Filarial						
Ag Negative	3370	90.8	1261	91.1	4631	90.0
Positive	342	9.2	123	8.9	465	9.1
Stool or Circulating Ag						
Negative	2276	61.3	897	64.8	3173	62.3
Positive	1436	38.7	487	35.2	1923	37.7

TABLE 5

DISTRIBUTION OF PPD REACTIVITY BY HELMINTH INFECTIONS

	PPD Reaction > 10						PPD Reaction > 12						Total	
	Missing		No		Yes		Missing		No		Yes			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Total	633	12.4	1927	37.8	2536	49.81	633	12.4	2244	44.0	2219	43.5	5096	100.0
Stool Helminth Infection														
Negative	434	12.5	1336	38.6	1695	48.9	434	12.5	1549	44.7	1482	42.8	3465	100.0
Positive	199	12.2	591	36.2	841	51.6	199	12.2	695	42.6	737	45.2	1631	100.0
Circulating Filarial Ag														
Negative	568	12.3	1776	38.4	2287	49.4	568	12.3	2062	44.5	2001	43.2	4631	100.0
Positive	65	14.0	151	32.5	249	53.5	65	14.0	182	39.1	218	46.9	465	100.0
Stool or Circulating Ag														
Negative	394	12.4	1234	38.9	1545	48.7	394	12.4	1426	44.9	1353	42.6	3173	100.0
Positive	239	12.4	693	36.0	991	51.5	239	12.4	818	42.5	866	45.0	1923	100.00

TABLE 6

DISTRIBUTION OF PPD REACTION SIZE BY HELMINTH INFECTIONS

	PPD Reaction Size			
	N	Mean	SE	Median
Total	4463	12.15	0.09	12.00
Stool Helminth Infection	3031	11.99	0.11	12.00
Negative				
Positive	1432	12.48	0.17	13.00
Circulating Filarial Ag	4063	12.10	0.10	12.00
Negative				
Positive	400	12.66	0.30	14.00
Stool or Circulating Ag	2779	11.96	0.12	12.00
Negative				
Positive	1684	12.46	0.15	13.00

TABLE 7

Factor	Category		STOOL HELMINTH INFECTION		CIRCULATING ANTIGENS		STOOL INFECTION OR CAg	
	A	B	Odds Ratio	95% CI	Odds Ratio	95% CI	Odds Ratio	95% CI
BCG SCAR	**Present**	**Absent**	**0.831**	**0.726, 0.951**	**0.961**	**0.775, 1.193**	**0.861**	**0.757, 0.979**

Odds ratio > 1 implies that patients in category A have higher risk of outcome compared with category B.

Bold odds ratio implies $p < 0.05$

TABLE 8

ANALYSIS OF RELATIONSHIP BETWEEN BCG SCAR AND HELMINTH INFECTION ADJUSTED FOR AGE AND GENDER

Factor	Category		STOOL HELMINTH INFECTION		CIRCULATING ANTIGENS		STOOL INFECTION ORCAg	
	A	B	Odds Ratio	95% CI	Odds Ratio	95% CI	Odds Ratio	95% CI
BCG SCAR	Present	Absent	0.808	0.703,0.929	0.972	0.775, 1.218	0.844	0.739, 0.965
GENDER	Male	Female	0.887	0.787, 0.999	1.529	1.261, 1.855	0.994	0.887, 1.115
AGE GROUP	(14-28] (28-42] >42	<14	1.008 1.123 1.317	0.851, 1.194 0.950, 1.328 1.114, 1.557	1.651 1.571 1.267	1.248, 2.183 1.188, 2.077 0.945, 1.700	1.117 1.196 1.301	0.950, 1.313 1.019,1.404 1.106, 1.530

Odds ratio > 1 implies that patients in category A have higher risk of outcome compared with category B.

Hold odds ratio implies $p < 0.05$

TABLE 9

UNIVARIATE ANALYSIS OF RELATIONSHIP BETWEEN PRESENCE OF HELMINTH INFECTION AND PPD REACTIVITY

Factor	Category		PPD REACTION > 10 mm		PPD REACTION > 12 mm	
	A	B	Odds Ratio	95% CI	Odds Ratio	95% CI
STOOL HELMINTH INFECTION	Positive	Negative	1.122	0.988, 1.274	1.108	0.977, 1.257
CIRCULATING ANTIGENS	Positive	Negative	**1.281**	**1.036,1.582**	**1.234**	**1.004, 1.516**
STOOL INFECTION OR CAg	Positive	Negative	**1.142**	**1.010,1.291**	1.116	0.989, 1.259

Odds ratio > 1 implies that patients in category A have higher risk of outcome compared with category B.

Bold odds ratio implies $p < 0.05$

TABLE 10 A

RELATIONSHIP BETWEEN PRESENCE OF STOOL HELMINTH INFECTION AND PPD REACTIVITY ADJUSTED FOR AGE AND GENDER

Factor	Category		PPD REACTION > 10 mm		PPD REACTION > 12 mm	
	A	B	Odds Ratio	95% CI	Odds Ratio	95% CI
STOOL HELMINTH INFECTION	**Positive**	**Negative**	**1.030**	**0.888, 1.195**	**1.023**	**0.886, 1.181**
GENDER	**Male**	**Female**	**1.641**	**1.423, 1.892**	**1.517**	**1.322,1.740**
AGE GROUP	**(14-28) (28-42) >42**	**514**	**6.397 16.844 17.680**	**5.280, 7.749 13.73, 20.67 14.34, 21.79**	**5.435 15.276 16.118**	**4.443, 6.648 12.43,18.77 13.07,19.88**

Odds ratio > 1 implies that patients in category A have higher risk of outcome compared with category B.

Bold odds ratio implies $p < 0.05$

TABLE 10 B

RELATIONSHIP BETWEEN PRESENCE OF CIRCULATING FILARIAL ANTIGEN AND PPD REACTIVITY ADJUSTED

Factor	Category		PPD REACTION > 10 mm		PPD REACTION > 12 mm	
	A	B	Odds Ratio	95% CI	Odds Ratio	95% CI
CIRCULATING ANTIGENS	Positive	Negative	1.108	0.867, 1.417	1.083	0.856, 1.370
GENDER	Male	Female	**1.634**	**1.417,1.885**	**1.511**	**1.317,1.734**
AGE GROUP	(14-28] (28-42) >42	514	**6.370** **16.799** **17.679**	**5.257, 7.719** **13.69, 20.61** **14.34, 21.79**	**5.416** **15.240** **16.114**	**4.426, 6.627** **12.40,18.73** **13.07,19.87**

Odds ratio > 1 implies that patients in category A have higher risk of outcome compared with category B.

Bold odds ratio implies $p < 0.05$

TABLE 10C

RELATIONSHIP BETWEEN PRESENCE STOOL HELMINTH INFECTION OR CIRCULATING FILARIAL ANTIGEN AND PPD REACTIVITY ADJUSTED FOR AGE AND GENDER

Factor	Category		PPD REACTION > 10 mm		PPD REACTION > 12 mm	
	A	B	Odds Ratio	95% CI	Odds Ratio	95% CI
STOOL INFECTION OR CIRCULATING ANTIGENS	Positive	Negative	1.041	0.903, 1.201	1.021	0.889, 1.173
GENDER	Male	Female	**1.640**	**1.422,1.891**	**1.516**	**1.322,1.739**
AGE GROUP	(14-28] (28-42] >42	514	**6.389** **16.831** **17.669**	**5.274, 7.741** **13.72, 20.65** **14.33, 21.78**	**5.432** **15.272** **16.118**	**4.440, 6.645** **12.43,18.77** **13.07,19.88**

Odds ratio > 1 implies that patients in category A have higher risk of outcome compared with category B.
Bold odds ratio implies $p < 0.05$

Annexure-2

Scientific / Participations / Presentations / Publications

INDIAN RED CROSS SOCIETY

Dr. S. Rajasekaran

has been enrolled as a

Life Member

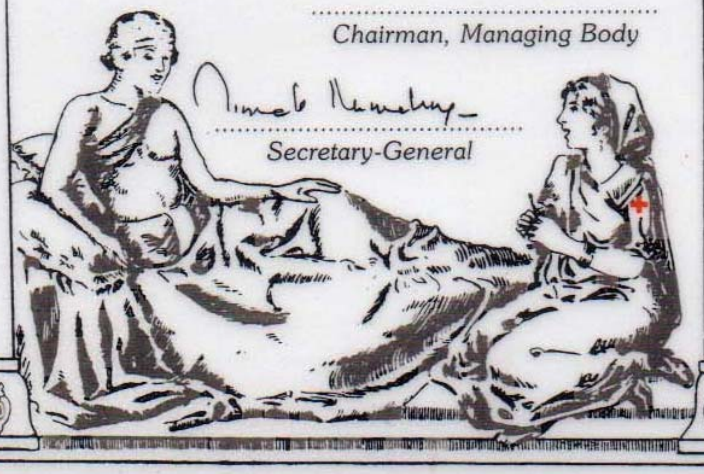
of the Society through the

Tamil Nadu State Branch

and in testimony thereof this certificate is signed and issued by us.

Chairman, Managing Body

Secretary-General



No. 6671 of 2000

11th International Congress of Immunology
Stockholm, Sweden, 22-28 July, 2001

This is to certify that

Sivaprakasam Rajasekaran

has attended the 11th International Congress of Immunology, Stockholm, July 22-27, 2001 and presented the abstract

Clinical Aspects of Lymphatic Filariasis

in the workshop 5.25 Immunity to Pathogens of the Developing World on Friday, July 27, 2001

Olli Vainio
Chairman of the Abstract Committee

11th International Congress of Immunology
22–27 July, 2001, Stockholm, Sweden

Congrès
Mondial
Dermatologie
Paris 2002

Jean Revuz
Président

Jean-Paul Ortonne
Secrétaire Général

Louis Dubertret
Vice-Président

Dr S Rajasekaran
Derm Center
10, Ritherdon Avenue
Vepery
6000 07 Chennai
India

Paris, July 2002

Dear Dr Rajasekaran,

On behalf of the International Committee of Dermatology and the Organising Committee of the 20th World Congress of Dermatology that was held recently in Paris, we would like to thank you very much for your contribution.

For your information, 12000 delegates and 1500 accompanying persons registered at the congress. 4500 (approximately) exhibitors were present. The attendance at the scientific sessions was excellent. As far as we know, the congress generated high satisfaction among participants.

Your contribution was determinant for the success of this world congress. Again we thank you very warmly for your active participation.

Best regards,

Jean Revuz

Jean-Paul Ortonne

Louis Dubertret

Congrès
Mondial
Dermatologie
Paris 2002

ATTENDANCE CERTIFICATE

Jean Revuz
Président

Jean-Paul Ortonne
Secrétaire Général

Louis Dubertret
Vice-Président

We hereby certify that : **Dr Rajasekaran SIVAPRAKASAM** attended the congress:

20th World Congress of Dermatology

from June 30th to July 5th 2002

Philippe Fournier

Registration N° D341/ 2965

Congress secrétariat 20th World Congress of Dermatology : co/**COLLOQUIUM** - 12, rue de la croix-Faubin - 75557 PARIS cedex 11
Tel : 33 (0)1 44 64 15 15 - Fax : 33 (0)1 44 64 15 16 & 17 - e-mail : p.fournier@colloquium.fr - Web site : www.derm-wcd-2002.com

Congrès
Mondial
Dermatologie
Paris 2002

CERTIFICATE OF ORAL PRESENTATION

We hereby certify that : **Dr Rajasekeran S.**

did present the communication referred **CC1159** (on 4 July 2002) :

A CASE OF DISSEMINATED CUTANEOUS RHINOSPORIDIOSIS AS A PRESENTING FEATURE OF AIDS

Yesudian P., Murugusundram S., Rajasundram S., Sampathkumar K.V., Rajasekeran S.
Derm Center, 10, Ritherdon Avenue, Vepery, Chennai-7, India

at the World Congress of Dermatology which was held in Paris, Palais des Congrès from June 30 to July 5, 2002

20th WORLD CONGRESS OF DERMATOLOGY
29 JUNE - 5 JULY 2002
c/o COLLOQUIUM
12 rue de la Croix Faubin - 75557 PARIS CEDEX 11 - FRANCE
Tel : 33 (0)1 44 64 15 15 - Fax : 33 (0)1 44 64 15 16 & 17

The Congress Scientific Secretariat

IUIS International Union of Immunological Societies

FOCiS Federation of Clinical Immunology Societies www.focisnet.org

This is to certify that

Sivaprakasam Rajasekaran

has attended
the 12th International Congress of Immunology
and 4th Annual Conference of the Federation of
Clinical Immunology Societies

held in Montréal, Canada from July 18 – 23, 2004

Emil Skamene, Congress President

International Union of Immunological Societies

FOCIS Federation of Clinical Immunology Societies

12th International Congress of Immunology and 4th Annual Conference of FOCIS

Poster Presentation

Dear Dr. Rajasekaran:

We are pleased to inform you that your abstract has been accepted for poster presentation at the 12th International Congress of Immunology and the 4th Annual Meeting of FOCIS to be held July 18–23, 2004, in Montréal, Canada. All abstracts will be published as a supplement to *Clinical Investigative Medicine*.

All posters will be on view for one day of the meeting, from 7:30 to 18:00 hours.

Abstract Confirmation Number: 3769
Abstract Title: A study of Parasite and Mycobacterial Coinfections.

Session: Infectious diseases – Parasitic I
Date: Tuesday, July 20, 2004
Location: Exhibit Hall Palais des congrés
Publication Number: T45.87
Poster Board Number: 726AM
Mount Time: 07:00 hours
Viewing Time: 07:30–18:00 hours
Presentation Time: – If AM appears after your poster board number, please stand by your poster from 07:30–08:30 hours

– If PM appears after your poster board number please stand by your poster from 12:30–13:30 hours

Dismount Time: **Immediately after your session**

Please Note: Any poster not taken down by 18:30 hours on the day of display will be discarded

For instructions on the preparation of your poster please see:

http://www.immuno2004.org/e/call_abstracts/postersessions_e.html

You should be in attendance for the entire presentation time noted above. Your poster board area is approximately 1.0 m high (approx. 39 inches) x 1.5 m (approx. 59 inches) wide and is made of a soft material that accepts Velcro tape. A supply of Velcro tape will be available in the poster area for mounting your poster. There will be a Poster Presenter check in desk located inside the Exhibit Hall of the Palais des congrès. Please pick up your Velcro tape at this counter on the morning of your poster presentation.

All presenters must register for the meeting. Early registration ends April 30. Complete registration information is available online at www.immuno2004.org

If you wish to withdraw your poster the deadline to withdraw is **May 1st, 2004**. Please complete the Abstract Withdrawal form and FAX to +1–507–334–0126 (attention Cassie Carver)

We look forward to seeing you in Montréal!

Sincerely yours,

Marianna Newkirk

Marianna Newkirk, Ph.D.
Chair of the Scientific Program Committee

AIDS 2006: Notification of CD-Rom

Inbox

AIDS 2006 Abstracts <abstracts More options May 3

Dear Sivaprakasam Rajasekaran,

Thank you for submitting your abstract entitled:

Consequences of multiple co-infections in areas endemic for lymphatic filariasis

to the XVI International AIDS Conference to be held in Toronto, Canada, 13-18 August 2006.

We received a huge response to our call for abstract submissions to this important conference and received many high quality abstracts. Each abstract has now been anonymously peer-reviewed by at least three members of the International Abstract Review Committee.

Based on the average score your abstract received, the Scientific Programme Committee has not been able to allocate space for your abstract as an oral presentation or a poster discussion. However, your contribution is appreciated and will be published on the conference CD-ROM, distributed at the conference, which is an extension of the Abstract Book. In addition, your contribution will also be published on the conference website.

Your abstract number is: CDC0237.

FURTHER INFORMATION

Should you need further information regarding your contribution, or should you wish to withdraw your abstract from publication on the CD, please contact the AIDS 2006 Abstract Team by email at abstracts@aids2006.org. Please note that the presenting author only may withdraw the abstract.

We would very much like to see you in Toronto. Registration for the conference can be made through http://www.aids2006.org. Please register before 15 May to avoid the late registration surcharge!

In order to facilitate the visa application process (list of Countries and Territories whose Citizens require visas in order to enter Canada as visitors can be found here http://www.cic.gc.ca/english/visit/visas.html), we will

5047

Buenos Aires XXI Congressus Mundi Dermatologiae 2007

21st World Congress of Dermatology

September 30 - October 5, 2007 Buenos Aires, Argentina

This is to certify that **S Rajasekaran, S Murugusundram, P Yesudian**

presented the Abstract **"MYSTERIOUS TATTOO PIGMENTATION OF THE FACE"**

in the **Poster Exhibit "Clinical dermatology: Pigmentary disorders"**

Buenos Aires, October 5, 2007

I.L.D.S. International League of Dermatological Societies

Adrián M. Pierini, MD
Secretary General

Ricardo L. Galimberti, MD
President

4704

XXI Congressus Mundi Dermatologiae
Buenos Aires 2007

21st World Congress of Dermatology

September 30 - October 5, 2007 Buenos Aires, Argentina

This is to certify that **S Rajasekaran, S Murugusundram, P Yesudian**

presented the Abstract **"PRETIBIAL MYXOEDEMA - AN IMPORTANT IMITATOR OF FILARIASIS IN THE TROPICS"**

in the **Poster Exhibit "Clinical dermatology: Internal medicine – collagen tissue diseases"**

Buenos Aires, October 5, 2007

I.L.D.S
International League of Dermatological Societies

Adrián M. Pierini, MD
Secretary General

Ricardo L. Galimberti, MD
President

14th ICI KANSAI 2010

JSI JSGI IUIS

Certificate of Attendance

We hereby certify that

has attended the Congress below:

The 14th International Congress of Immunology

held in Kobe, Japan

August 22 - 27, 2010

T. Kishimoto

Tadamitsu Kishimoto, M.D., Ph.D.

President of the 14th International Congress of Immunology

Please select Print from the file menu to print your Abstract.

12th International Congress of Immunology and 4th Annual Conference of FOCIS

Filename: 955597

Presenting Author: Sivaprakasam Rajasekaran

Author for Correspondence Sivaprakasam Rajasekaran, Dr

Department/Institution: Immunology, Tuberculosis Research Centre

Address: Spur Tank Road, Chetput

City/State/Zip/Country: Chennai, Tamil Nadu, 600 031, India

Phone: 91-44-28362432 **Fax:** 91-44-28362528 **E-mail:** kumarav@hotmail.com

Abstract Categories: Infectious diseases - Parasitic - Helminths

FOCIS Award: Please do not consider me for a FOCIS New Investigator Travel award.

EFIS Award: Please do not consider me for a EFIS New Investigator Travel award.

Presentation format: Either Poster or Platform

Marketplace interest: Yes

Title: A study of parasite and mycobacterial coinfections

Sivaprakasam Rajasekaran, PhD [1] and Vasanthapuram Kumaraswami, MD PhD [1]. [1] Immunology, Tuberculosis Research Centre, Chennai, Tamil Nadu, India, 600 031 .

Lymphatic filariasis is a major health public problem in many tropical countries of the world. An estimated 1.2 billion people are at "risk" of developing the disease and nearly 120 million have one form of the disease or other. While the clinical manifestations are well documented the psychosocial damage caused by the disease is poorly understand. The immune responses that drive the disease are predominantly of the Th2 class which are characterized by elevated levels of IL4 and IL5 and eosinophils. In these endemic areas co-infections with viruses (HIV), bacteria (tuberculosis) and other parasites (geohelminths) are common. Protective immune responses directed against these organisms are predominantly of the Th1 sub types. Recent experimental studies suggest that Th2 responses generated by parasites may interfere with the protective immune response directed against invading intracellular parasites. However, the frequency of the occurrence of these co-infections in large populations has not been well studied. We examined the prevalence of co-infections by M. tuberculosis and helminthiasis in a W.bancrofti filariasis endemic area in South India to evaluate the influence of parasites on the immune responses to M. tuberculosis. Individuals were screened for the presence of tuberculosis infection by skin testing, sputum examination and radiological examinations. The presence of geohelminths was detected by stool examination while the presence of filarial infection was documented by screening the population using card tests for the detection of circulating filarial antigens. Coinfections of mycobacteria and helminths were common in this study area. The clearance of the parasite infection on the mycobacterial responses will be presented and discussed.

Signature of Presenting Author:

Manuscript Accepted in Lymphology, 2016, #1503/16

Multi modality treatment for the reversal of Grade IV lymphedema – a Case Study

Rajasekaran Sivaprakasam[1], Rajamanickam Anuradha[2], Ramalingam Bethunaickan[1], and Gurusamy Manoharan [3, #]

1 Department of Immunology, National Institute for Research in Tuberculosis, Chennai -India

2 International Centre for Excellence in Research - National Institute of Health – National Institute for Research in Tuberculosis, Chennai, India.

3 Department of Plastic surgery, Apollo Hospitals, Chennai – India.

#Address Correspondence to:

Dr. G.Manoharan, MS. MCh, FICS (Plastic).,

Senior consultant Plastic & Reconstructive surgeon & lymphologist,

21 Greams Road, Chennai -600 006

Telephone: +91 44 2829 6580

Fax: +91 44 2829 4429

E-mail address: gmano.dr@gmail.com

Key words: Grade IV Lymphedema, Multi modality Treatment, Nodo venal shunt.

Multi modality treatment for the reversal of Grade IV lymphedema- a case study

Here we present, a case study of 37 year old female, admitted with giant lymphedema (Grade IV) for 27 year duration (Fig. 1). Preoperative lymphoscintigraphy picture revealed multiple lymphatic channels, with huge dermal backflow and multiple inguinal lymph nodes. (Fig. 2). For which multi modality treatment and treated successfully with an aesthetically and functionally acceptable lower limb. This case was unique for its size and presentation of the right lower limb. After getting initial basic foot care in the form of daily cleaning with Betadine Scrub, antifungal powder together with foot elevation with a course of oral penicillin (800mg/twice daily for one week), the patient was taken for Manual Lymph Drainage (MLD) and multi layer bandaging for ten days. This was done twice daily with respiratory physiotherapy and walking.

Earlier experience from our surgical intervention towards grade I and grade II for its reversal had empowered us to include this surgical correction as our choice in the multimodality treatment. (B.-B Lee et al., Lymphedema.2011). Hence we performed a new technique of superficial excision of alternate lumps in three stages at the interval of 6 weeks. Then we carried out Nodovenal shunt to retain the achieved results and avoid recurrence of this swelling. This multi modality treatment for this patient has given the best outcome in cosmetic and functional result (Fig. 3).

This patient was followed for the past ten years without any recurrences or complications. Following this we started and recommend multimodality treatment in grade III and IV lymphedema patient as a regular practice of morbidity control of lymphatic filariasis. This case is presented here for its uniqueness in terms of presentation and the outcome we have achieved using multimodality treatment in lymphatic filariasis.

Reference:

1. B.-B et.al., Lymphedema, A concise compendium of theory and practice. 2011. (C)Springer – Verlag London Ltd. ISBN: 978-0-85729-5668.

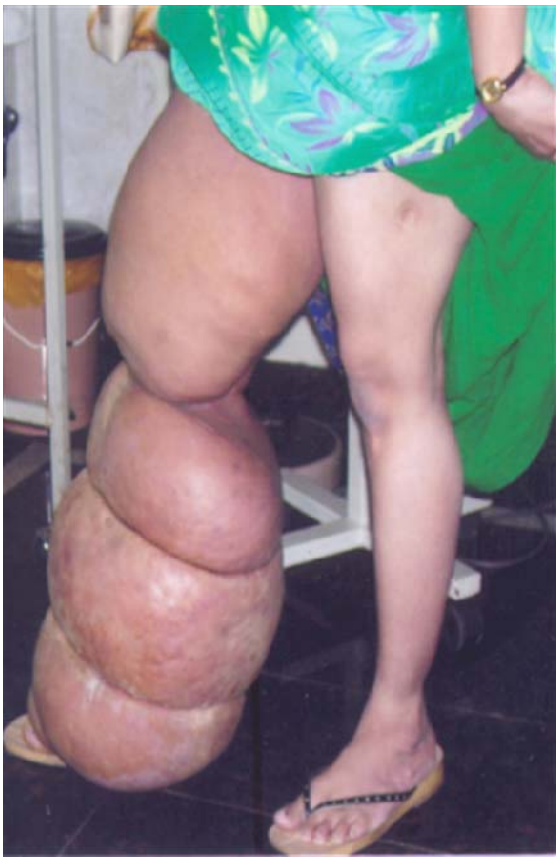


Fig.1. Giant lymphedema (Grade IV -Right leg)

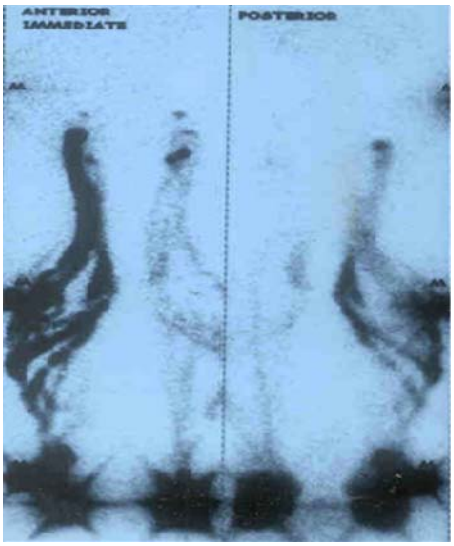


Fig. 2. Pre operative lymphoscintigraphy picture showing multiple lymphatic channels, with huge dermal backflow at the later pictures and multiple inguinal lymph nodes.

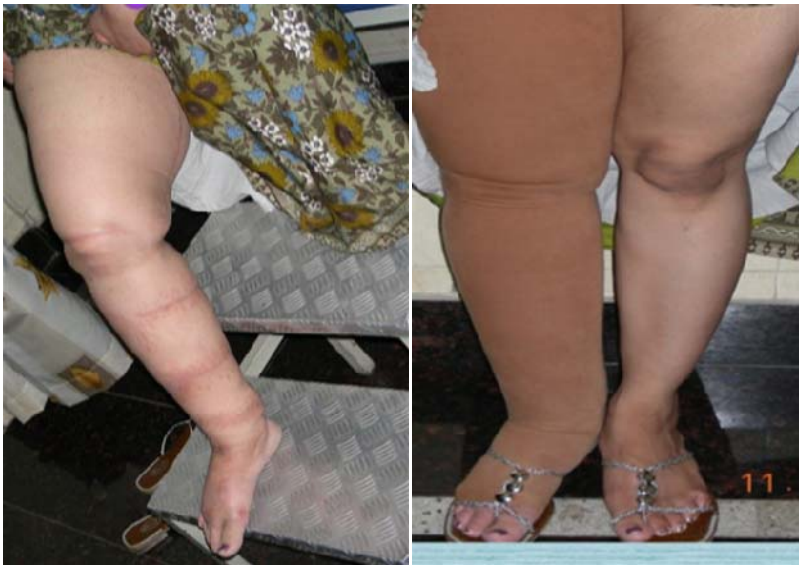


Fig. 3. Reversed Grade IV lymphedema, after multi modality treatment (Manual Lymph Drainage, Multilayer bandaging and Surgical Intervention (Nodovenal Shunt).

Manuscript Accepted in Lymphology, 2016, #1506/16

Recent advances and clinical management in Lymphatic Filariasis

Rajasekaran Sivaprakasam[1], Rajamanickam Anuradha[2], Gurusamy Manokaran[3] and Ramalingam Bethunaickan[1, #]

1. Department of Immunology, National Institute for Research in Tuberculosis, Chennai –India
2. International Center for Excellence in Research - National Institute of Health – National Institute for Research in Tuberculosis, Chennai, India.
3. Department of Plastic surgery, Apollo Hospitals, Chennai – India.
Address Correspondence to:

Dr. Ramalingam Bethunaickan, Ph.D.,
Scientist D & DBT Ramalingaswami Fellow,
Department of Immunology,
National Institute for Research in Tuberculosis (ICMR),
No.1. Mayor Satyamoorthy Road,
Chetpet, Chennai- 600 031.
Telephone : +91 44 2836 9761
Fax : +91 44 2836 2525
E-mail address: bramalingam@gmail.com

Key words: Lymphedema, Clinical management, Lymphatic Filariasis,

Recent advances and clinical management in Lymphatic Filariasis

Abstract

Filariasis is caused by thread-like nematode worms, classified according to their presence in the vertebrate host.The lymphatic group includes *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori.* Lymphatic filariasis, a mosquito-borne disease, has been one of the most prevalent diseases in tropical and subtropical countries and is accompanied by a number of pathological conditions. It is estimated that, currently, *i.e.* after 13 years of the MDA programme, there are still an estimated 67.88 million LF cases that include 36.45 million microfilaria carriers, 19.43 million hydrocele cases and 16.68 million lymphedema cases. [Ramaiah et al-2014 and Hooper et al 2014] Although two-thirds of the 120 million people infected with lymph-dwelling filarial parasites have subclinical infections, ~ 40 million have lymphedema and/or other pathologic manifestations including hydroceles (and other forms of urogenital disease), episodic adenolymphangitis, tropical pulmonary eosinophilia, lymphedema, and (in its most severe form) elephantiasis. Adult filarial worms reside in the lymphatics and lymph nodes and induce changes that result in dilatation of lymphatics and thickening of the lymphatic vessel walls. Progressive lymphatic damage and pathology results from the summation of the effect of tissue alterations induced by both living and nonliving adult parasites. In recent years, there has been rapid progress in filariasis research, which has provided new insights into the pathogenesis of filarial disease, diagnosis, chemotherapy, the host–parasite relationship and the genomics of the parasite. This review discuss about the clinical manifestations of the disease, diagnosis and treatment, immune responses, its management are discussed along with a review on drugs against filariasis in this article. Detail on the infection, safety profile, and status in clinical practices are also covered.

Introduction

The term “lymphatic filariasis” comprises infection with three closely related nematode worms – *Wuchereria bancrofti, Brugia malayi and Brugia timori*. All three parasites are transmitted by the bites of infective mosquitoes and have quite similar life cycles in humans with the adult worms living in the afferent lymphatic vessels while their progeny, the microfilariae, circulate in the peripheral blood and are available to infect mosquito vectors when they feed.

Lymphatic filariasis (LF) is a neglected mosquito-borne tropical disease. It is endemic in 58 countries, it was estimated that 1.2 billion people at risk globally with an estimated 120 million infected. It is the second leading cause of permanent or long-term disability with over 40 million infected people suffering from pathological manifestations like lymphoedema, hydrocoele, chyluria and elephantiasis [Organization, W. H. Lymphatic filariasis]. In India LF is endemic in 255 districts of 16 states and 5 Union Territories (UTs) of the country. Presently about 630 million of people in these endemic states/UTs are at-risk of LF. [**AC Dhariwal et al 2015]**

LF is responsible for 6.3 million disability adjusted life years (DALYs) [Hotez et al., 2009; Hotez and Kamath, 2009; WHO, 2011]. These infections also lead to increased mortality [Kirkwood et al., 1983; Little et al., 2004; Pion et al., 2002; Walker et al., 2012]. In addition to the economic loss due to lost productivity, there are negative social and cultural effects of the diseases [Evans et al., 1993]. Elimination of lymphatic filariasis is possible by interrupting the transmission cycle. Providing treatment on a large-scale to entire communities where the infection is present can stop the spread of infection. This strategy of preventive chemotherapy, called mass drug administration (MDA), involves a combined dose of 2 medicines given annually to an entire at-risk population in the following way: albendazole (400 mg) together with ivermectin (150–200 mcg/kg) or with diethylcarbamazine citrate (DEC) (6 mg/kg). These medicines have a limited effect on adult parasites but effectively reduce microfilariae from the bloodstream and prevent the spread of microfilaria to mosquitoes.[F. SchŠberlea, et al 2014]

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

Clinical manifestations

The clinical manifestations of LF are varied, Traditionally, it has been accepted that people living in an endemic area can be classified into five groups: (1) Endemic Normals; (2) clinically asymptomatic, infected; (3) Acute clinical disease; (4) Chronic pathology and (5) Tropical pulmonary eosinophilia (TPE).

Endemic Normals

In an endemic area, a proportion of the population remains uninfected despite exposure to the parasite. This group has been termed as endemic normals

Asymptomatic patent infection

In areas endemic for lymphatic filariasis, many individuals exhibit no symptoms of filarial infection

and yet, on routine blood examinations, demonstrate the presence of significant numbers of parasites or the presence of circulating parasite antigen (a surrogate for viable adult worms). These individuals are carriers of infection. With the availability of better imaging techniques (e.g., ultrasound, lymphoscintigraphy, MRI, CT), it has become apparent that almost everyone with active infection (e.g., microfilarial positivity) has some degree of lymphatic abnormality that may include: dilatation and tortuosity of lymph vessels with collateralization, increased or abnormal patterns of lymph flow [Freedman DO et al 1994,1995] and urogenital lymphangiectasia [Noroes et al 1996,1996] and microscopic hematuria and/or proteinuria [Dreyer G et al 1992]. At least half of all patients with lymphatic filariasis appear clinically asymptomatic. This asymptomatic presentation exists despite the presence of microfilariae in their blood and hidden damage to their lymphatics. [Kabatereine NB et al 2010,].

Acute clinical Disease

Acute manifestations of lymphatic filariasis are episodic attacks of lymphadenitis and lymphangitis (fever, pain in the affected part, tender red streaks) along with fever and malaise. Over 90% of cases with chronic manifestations will give a history of acute attacks. During acute infection the microfilariae, larval forms and are transmitted by mosquitoes of various species. Occasionally the adult worms and their associated granulomatous reaction are manifested as lumps in the subcutaneous tissue, breasts or testicles [Kabatereine NB et al 2010,Narain JP et al 2010, Al-Shaham AA et al 2010]. Acute filariasis is characterized by episodic occurrence of inflammation of the lymph glands (lymphadenitis), inflammation of the lymph channels (lymphangitis) and subsequent swelling of the limbs or scrotum (lymphedema).Filariatic fever is often seen with headache and chills, and will usually occur at the same time as lymphangitis Lymphadenitis and lymphangitis are characteristic of both the *W. bancrofti and B. malayi* forms [Dreyer G et al 1992]. The lymph nodes commonly involved are the inguinal, axillary and epitrochlear nodes and, in addition, the lymphatic system of the male genitals are frequently affected in *W. bancrofti* infection leading to funiculitis, epididymitis and/or orchitis [Pani SP et al 1995]. The Funiculo-epididymoorchitis, lymphadenitis and retrograde lymphangitis has been termed acute dermatotolymphangitis, a process characterized by development of cutaneous or sub-cutaneous inflammation and accompanied by ascending lymphangitis and regional lymphadenitis. This manifestation is thought to result primarily from bacterial and fungal superinfections of the affected limbs [Dreyer G et al 1992].

Lymphadenitis and lymphangitis are characteristic of both the *W. bancrofti* and *B. malayi* forms [Okon OE et al 2010]. In lymphadenitis, the parasite essentially lodges inside the lymph nodes in the body, causing immune reaction and inflammation. Blockage and stretching of the lymph vessels by the adult worms make it difficult for lymph to flow out of the lymphatics and back into the blood stream. Inflammation of the lymph channels and lymph nodes along with a decreased draining efficiency leads to lymphedema. Lymphatic trunks become very painful and the skin on the arms and legs may show red streaks from the infected lymphatics. The distal end of the affected limb becomes swollen during the attack and remains swollen for several days. Usually the swelling is initially limited to a single limb.

Chronic pathology

The chronic pathology of lymphatic filariasis develops years after initial infection [Partono F et al 1987]. The most commonly affected nodes are in the femoral and epitrochlear regions. Abscess formation may occur at the nodes or anywhere along the distal vessel. Infection with *Brugia timori* (*B. timori*) appears to result in more abscesses than infection with *B. malayi* [Edeson JF et al 1962] or *W. bancrofti* [okon et al 2010]. The granulomas are characterized by macrophages (which develop into giant cells), with plasma cells, eosinophils, neutrophils and lymphocytes and with hyperplasia of the lymphatic endothelium, occur with repeated inflammatory episodes. The consequence is lymphatic damage and chronic leakage of protein-rich lymph in the tissues, thickening and verrucous changes of the skin, and chronic bacterial and fungal infections, which all contribute to the appearance of elephantiasis. *B.malayi* elephantiasis is more likely to affect the upper and lower limbs, with genital pathology and chyluria being rare.

Stages of Lymphedema of the Leg (Stage I)

- Swelling reverses at night
- Skin folds-Absent
- Appearance of Skin-Smooth, Normal

Stage II

- Swelling not reversible at night
- Skin folds-Absent
- Appearance of skin-Smooth, Normal

Stage III

- Swelling not reversible at night
- Skin folds-Shallow
- Appearance of skin-Smooth, Normal

Stage IV

- Swelling not reversible at night
- Skin folds-Shallow
- Appearance of skin - Irregular,
- * Knobs, Nodules

Stage V

- Swelling not reversible at night
- Skin folds-Deep
- Appearance of skin – Smooth or Irregular

Stage VI

- Swelling not reversible at night
- Skin folds-Absent, Shallow, Deep
- Appearance of skin *Wart-like lesions on foot or top of the toes

Stage VII

- Swelling not reversible at night
- Skin folds-Deep
- Appearance of skin-Irregular
- Needs help for daily activities - Walking, bathing, using bathrooms, dependent on family or health care systems

Tropical Pulmonary Eosinophilia

Tropical pulmonary eosinophilia (TPE) is a distinct syndrome that develops in some individuals infected with *W. bancrofti and B. malayi* [Ong RK,et al 1998, Ottesen EA et al 1992]. Tropical pulmonary eosinophilia is an extreme immune response to filarial infection. High eosinophilia levels, asthma-like symptoms and restrictive lung disease are characteristics of TPE. This manifestation occurs with low frequency in endemic areas. Chest x-rays may be normal but generally show increased bronchovascular markings; diffuse miliary lesions or mottled opacities may be present in the middle and lower lung fields. Total serum IgE levels (10,000 to 100,000 ng/mL) and antifilarial antibody titers are characteristically elevated.

Diagnosis of Lymphatic Filariasis

Diagnosis of LF was once an extremely challenging task but with the advent of recent antigen-detection techniques, such as ICT card test and ELISA-based on the Og4C3 monoclonal antibody, diagnosis has become much easier. Molecular xenomonitoring (MX), which detects filarial DNA in mosquitoes by PCR, is a highly sensitive assay. Ultrasonography (USG) and lymphoscintigraphy also revolutionized the diagnosis of the disease and may be very helpful in monitoring the success of chemotherapy. In TPE, serum antibodies like IgG & IgE will be extremely high and the presence of IgG4 antibodies indicate active infection. In brief here we have explained the few techniques

1. New techniques for antigen detection represent the highest quality lab test for diagnosing infection by *W.bancrofti*. PCR tests are also of high specificity and sensitivity, and detect parasite DNA in microfilariae in the blood in human those who have mf as well as in vectors in both bancroftian and brugian filariasis [McCarthy J, 2000]. Very high levels of specific IgG4 antibody in microfilaraemic patients have also been considered as a good diagnostic marker.
2. Immunochromatographic test (ICT) and Filaria Strip Test (FST), which are highly sensitive and specific filarial antigen detection assays, are available for the diagnosis of *W. bancrofti* infection [Weil G et al, 1997; Weil et al 2015]. With these tests, the parasite antigens can be detected independent of the microfilariae's periodicity. It is rapid (1-10 minutes), and no such test exists for Brugian filariasis. ELISA-based assay using the Og4C3 monoclonal antibody is equally sensitive and specific for detecting antigen in bancroftian infections.
3. Basic parasitologic testing of peripheral blood for microfilariae remains a diagnostic standby, keeping the periodicity of the microfilariae in mind [McCarthyJ, 2000].
4. Ultrasonography using a 7.5 or 10 MHz probe has helped to locate and visualise the movements of living adult filarial worms of *W. bancrofti* principally in the scrotal lymphatics of asymptomatic males with microfilaraemia [Amaral F et al, 1994, Anitha et al, 2001]
5. Lymphoscintigraphy has been found useful in tracing lymphatic damage, dermal backflow after injecting radiolabeled proteins intradermally in both symptomatic and asymptomatic infections [Freedmen DO et al 1995].

Pathogenesis of Filarial Disease

The most severe clinical manifestations of lymphatic filariasis are lymphedema and elephantiasis. Although the immune responses to filarial parasites have been well studied with respect to natural history, diagnosis and treatment, there is a relative paucity of information in terms of the mechanisms underlying development of pathology.

The two major independent components of lymphatic filarial disease are lymphangiectasia and inflammatory reactions. While most infected individuals exhibit lymphangiectasia, clinically apparent lymphedema may not be common [Freedman DO et al, Dreyer G et al, 2000]. It is also clear that with patent infection, lymphangiectasia develops in the vicinity of adult worm nests [Dreyer G et al, 2000]. Subclinical lymphangiectasia of the lymphatic vessels containing live adult worms have been shown to exhibit distention with no apparent inflammatory reactions in the vessel wall, with little or only a fleeting inflammatory response to living adult parasites [Dreyer G et al, 1999]. Further, the fact that lymphangiectasia is not restricted to the exact segment of lymphatics where the worms reside [Amaral F et al, 1994,Dreyer G, et al, 1994] suggests that this process is mediated by soluble products excreted or secreted by the parasite that act on the lymphatic endothelial cells. It is also clear that with the advent of adaptive immunity, the host inflammatory response against the dead or dying worm and the subsequent release of parasite products and inflammatory mediators, a stage of irreversible lymphatic dysfunction ensues [Figueredo-Silva J et al 2002, Connor DH et al 1986, von Lichtenberg F et al 1997]. This then manifests clinically as progressive lymphedema. In addition, lymphatic dysfunction has been shown to predispose infected individuals to secondary bacterial and fungal infections and trigger inflammatory reactions in the skin and subcutaneous tissue that accelerates the progression of lymphedema and precipitates the development of elephantiasis [Olszewski WL et al 1997, Shenoy RK et al 1999].

Cells of the innate and adaptive immune system are important for the initiation of type 2 immunity, which are the hallmark of helminth infections. The key players in T helper (Th) 2-type immunity are CD4+ Th2 cells and involve the cytokines—IL-4, IL-5, IL-9, IL-10, and IL-13; the antibody isotypes—IgG1, IgG4, and IgE, and expanded populations of eosinophils, basophils, mast cells, and alternatively activated macrophages [Allen JE and Maizels RM 2011,McSorley HJ, Maizels RM 2012].

The importance of pro-inflammatory cytokines, possibly of innate origin, in the pathogenesis of lymphedema, has been strengthened by a series of studies in humans with chronic pathology, either in early or late stages or lymphedema. Studies have shown that individuals with chronic lymphatic pathology have elevated levels of C-reactive protein (an acute phase protein, indicating an acute inflammatory response) [Lal RB et al 1991] pro-inflammatory cytokines such as TNF-α, IL-6 and soluble TNF receptor (Das BK et al 1996, Satapathy AK et al 2006), endothelin-1 and IL-2 (el-Sharkawy IM et al 2001), as well as IL-8, MIP-1α, MIP-1β, MCP-1, TARC and IP-10 (75) in the peripheral circulation. Similarly, while patients with both acute and chronic manifestations of LF have elevated circulating levels of IL-6 and IL-8, only those with chronic disease manifestations have elevated levels of sTNF receptors [Babu S et al 2005].

The endothelium appears to be closely associated with pathogenesis of lymphatic disease, studies targeting the interaction between endothelial cells (vascular or lymphatic) and filarial parasites have been performed. Differentiation of LEC into tube-like networks was found to be associated with significantly increased levels of matrix metalloproteinases (MMPs) and inhibition of their endogenous inhibitors—TIMPs (tissue inhibitors of MPs). [Bennuru S et al 2009]. Recent data suggest that an increase in circulating levels of MMPs and TIMPs is characteristic of the filarial disease process and that altered ratios of MMP/TIMP are an important underlying factor in the pathogenesis of tissue fibrosis in filarial lymphatic disease. [Anuradha R et al 2009] Other studies have implicated the vascular endothelial growth factor (VEGF) family in lymphangiogenesis. [Pfarr KM et al 2009, Debrah et al 2006] Other angiogenic factors such as angiopoietins-1 and -2 are also found at elevated levels in individuals with filarial-induced pathology. [Bennuru S et al 2010], A major factor involved in the initiation of the proinflammatory response and the increased production of VEGF-A and -C might be the endosymbiont, Wolbachia, present in most filarial nematodes (including W. bancrofti and the 2 Brugia spp) [Pfarr KM et al 2009]. Recently, it has been demonstrated that the increased levels of VEGF-C and sVEGF-R3 (observed in lymphedema patients) were reduced following doxycycline treatment (a regimen that eliminates Wolbachia) and that there was improvement in lymphedema. [Debrah et al 2006].

Persistent immune activation is associated with elevations of circulating microbial products, acute-phase proteins, and the so-called microbial translocation molecules. [Brenchley JM et al 2012] however, intra and peri-lymphatic damage—an underlying feature of filarial disease—might also contribute to the presence of microbial translocation products in the bloodstream. Indeed, the increased circulating levels of LPS (which serves as a marker for microbial translocation) and decreased levels of LPS-binding protein (LBP) are characteristic features of filarial lymphatic pathology [Anuradha R et al 2012] that in turn appears to cause immune activation. Since filarial lymphedema is known to be associated with increased bacterial and fungal loads in the lymphatics, our studies reveal that these damaged lymphatics may serve as a potential nidus for bacterial translocation through leaky lymphatic endothelium.

Multi-color flow cytometry analysis reveals that the frequency of Th1 cells (CD4+ T cells expressing either IFNγ or IL-2 or TNF-α) [Anuradha et al 2014]; Th9 cells (CD4+ T cells expressing IL-9 and IL-10) [Anuradha et al 2013]; Th17 cells (CD4+ T cells expressing IL-17) [Anuradha et al 2014] and Th2 cells (CD4+ T cells expressing IL-22) is significantly enhanced in filarial pathology. This is accompanied by a concomitant decrease in the frequency of Th2 cells (CD4+ T cells expressing IL-4 or IL-5 or IL-13) both at homeostasis and following parasite antigen stimulation [Anuradha et al 2014]. Although less well studied than Th1 cells, Th17 cells might also have an important role in the pathogenesis of disease in filarial infection since PBMC from individuals with pathology (but not asymptomatic patients) express significantly higher levels of the Th17 associated cytokines as well as the master transcription factor - RORC at the mRNA level [Babu S et al 2009].

Finally, pathology in lymphatic filariasis is also associated with expanded frequencies of Th9 cells, CD4+ T cells that express both IL-9 and IL-10 but not IL-4 and this frequency exhibits a positive correlation with the severity of lymphedema in filarial infections [Anuradha et al 2014].

Elimination programme and Treatment

The Global Program to Eliminate Lymphatic Filariasis (GPELF) recently released their progress report for 2014 [WHO Report Weekly epidemiological record 2015]. The report summarized the work of the GPELF's first decade, which was focused on implementing mass drug administration (MDA) across all LF endemic regions. The report acknowledged that whilst MDA programmes have been particularly successful in reducing infection within communities, efforts to reduce morbidity associated with LF remain lacking. Currently, only 24 of the 73 endemic countries have morbidity programs [WHO Report Weekly epidemiological record 2015]. These programs focus on hygiene, skin care, hydrocele surgery, and exercises [Dreyer G et al 2002]. The GPELF plan for 2010–2020 highlights the need for the establishment of morbidity management programs in all endemic regions. In particular, the plan identifies the need for the development of metrics to monitor and report on the outcomes of these programs [WHO Report Weekly epidemiological record 2015].

The National Filaria Control Programme (NFCP) was launched in India in 1955. The control strategy was selective chemotherapy with Diethylcarbamazine citrate (DEC) for 12 days at 6-mg/kg-body wt. for parasite carriers detected from the night blood survey, and larval control of vector mosquitoes. The major constraint of the NFCP was that it did not cover the vast majority of the population at risk residing in rural areas and that the strategy demanded detection of parasite carriers by night blood survey, which is less sensitive, expensive, time-consuming and poorly accepted by the community. **[**Sabesan S et al 2000].To eliminate lymphatic filariasis, a disabling and disfiguring neglected tropic disease also known as elephantiasis, the Indian Government has launched a substantial public health initiative in which they provide free prophylactic drugs to more than 400 million people in the country. The initiative includes providing an annual dose of preventive drugs (diethylcarbamazine and albendazole) to entire communities in the form of mass drug administration. To support this initiative, India's Ministry of Health and Family Welfare in collaboration with the Global Network for Neglected Tropical Diseases (an initiative of the Washington-based Sabin Vaccine Institute) has launched a public service advertising campaign called *Hathipaon Mukt Bharat* (Filaria Free India). The campaign includes a film entitled *Giant footprints!* In which a patient with lymphatic filariasis is shown delivering the message that the disease "can happen to anyone", that people should participate in the mass drug administration initiative, and that they should take the preventive medicines (which are free and safe) to make India fi laria free. The campaign support sthe Indian Government's mass drug administration initiative, which is being implemented in 17 states [Sanjeet Bagcchi reports April 2015]

Therapy for Lymphatic Filariasis

Remarkable advances in the treatment of LF have recently been achieved focusing not on individuals but on communities with infection, with the goal of reducing mf in the community, to levels below which successful transmission will not occur.

Chemotherapy of Filariasis

Drugs effective against filarial parasites

1. Diethyl Carbamazine citrate (DEC)
2. DEC-Fortified salt
3. Ivermectin
4. Albendazole
5. Levamisole hydrochloride
6. Moxidectin

Treatment of microfilaraemic patients may prevent transmission of infection and may be repeated every 6 months till mf and/or symptoms disappears.

Treatment and Prevention of ADL

The most distressing aspect of LF is the acute attacks of ADL, which result in considerable economic loss and deterioration of quality of life. Prompt treatment and prevention of ADL are of paramount importance. ADL may be seen both in early & late stages of the disease. It is due to the infection & inflammation of the skin and affected area due to entry of bacteria or fungus through the entry lesions. The skin becomes warm, tender, painful, swollen, and red. Patient develops fever, headache, chills and sometimes nausea and vomiting. Occasionally becomes septicemic. First sign will be enlarged, tender and painful swollen of L.nodes appears later lasting for 4-5days. Peeling & darkening of skin is common. Repeated attacks increase the size of the legs. Management includes symptomatic treatment like relieving pain, antibiotics to combat bacterial infection, care of entry lesions etc. In patients with late stages of oedema, long term antibiotic therapy using oral Penicillin or long acting parentral Benzathil Penicillin are sometimes used to prevent ADL.

Surgical Treatment

In the surgical aspect of lymphatic filariasis, grade I and grade II can be treated conservatively, whereas grade III and grade IV needs surgical correction together with regular antibiotic, chemotherapy with DEC. The old surgical techniques of excision and skin grafting is no more practiced as it gives poor cosmetic results, along with early recurrences. Thompson's, kondolean and Charles procedures are now given up and now more of newer techniques involving microvascular surgery like nodovenal shunt, lymphovenal shunt, with reduction and sculpturing is being carried out without skin grafting or a flap cover. Patient's local skin itself has been salvaged and made to a better quality by manual lymph drainage(MLD) , bandaging and use the same skin for reconstitution.(Manokaran G:2011)

Herbal treatments

There are several herbs that have been prescribed by Ayurveda for the treatment of elephantiasis for centuries. The following are some of the herbs reported as having antifilarial activities ie. Vitex negundo L. (roots)[Sahare KN et al 2008], Butea monosperma L. (roots and leaves)[Sahare KN et al 2008], Ricinus communis L. (leaves)[Sahare KN et al 2008], Aegle marmelos Corr. (leaves)[Sahare KN et al 2008], Canthium mannii (Rubiaceae)[Wabo Pone J et al 2009], Boerhaavia diffusa L. (whole plant) [Jain SP et al 2010]. Two compounds, oleanonic acid and oleanolic acid, isolated from hexane and chloroform fractions *in vitro* killed adult *B. malayi* [Misra N et al 2007]

Treatment and Prevention of Lymphedema and Elephantiasis

Early treatment with drugs may destroy the adult worms and logically prevent the later development of lymphoedema. Once lymphoedema is established there is often no complete cure, but the "foot care programme" may offer relief, some amelioration, prevention of acute attacks and thus limitation of further progression of the swelling.

Current Control Strategy of LF

In view of achieving the global elimination of LF, the programme in India has been made a part of the NVBDCP in 2003, under the National Health Policy 2002, and set a target for elimination of LF by 2015. The strategy for achieving this goal was initially by annual MDA single dose DEC (6 mg/kg body wt.) for at least five years to the entire population of an endemic district (excluding children under two years, pregnant women and severely ill patients), and home-based management of lymphoedema cases and hydrocelectomy operations in identified Community Health Centres (CHCs) and hospitals.

MDA with DEC was launched as a pilot project in 13 districts of seven states in the year 1996. [**http://www.namp.gov.in/filariasis.html].** The NVBDCP up-scaled the MDA to cover a population of 77 million in 2002 from

41million in1996-97. During the year 2004, a population of about 468 million from 202 districts was targeted for MDA. There have been several reviews of the use of Albendazole (Alb) for MDA towards the elimination of LF. [Pani SP et al 2002, Shenoy RK 1999, John O Gyapong et al 2005, Shenoy RK et al 2006, Sabesan et al 2006] A large-scale trial on the feasibility and impact of co-administration of DEC and Alb in selected districts in the country was carried out in 2000-05, with the support of Indian Council of Medical Research (ICMR) Task Force. It therefore recommended the co-administration (DEC 6 mg/kg/ body wt. and Alb 400 mg) strategy for all endemic districts in India.

Disease Management

Filarial patients with skin lesions often have more bacteria on the skin than usual. The large number of bacteria on the skin, multiple skin lesions, slow lymph fluid movement from the damaged lymphatics and the reduced ability of the lymph nodes to filter the bacteria cause inflammation characteristic of an acute attack. Repeated bacterial infections precipitate frequent acute attacks, which further damage the tiny lymphatic vessels in the skin, reducing their ability to drain fluid. This vicious cycle continues, aggravating the condition of the patient.

The lymphedema management involves the following components:

- ✓ Washing,
- ✓ Prevention and cure of entry lesions,
- ✓ Elevation of the foot,
- ✓ Exercise
- ✓ Wearing proper footwear,
- ✓ Management of acute attacks.

The GPELF aims to provide access to a minimum package of care for every person with associated chronic manifestations of lymphatic filariasis in all areas where the disease is present, thus alleviating suffering and promoting improvement in their quality of life.

Success in 2020 will be achieved if patients have access to the following minimum package of care:

- ➢ Treatment for episodes of adenolymphangitis (ADL);
- ➢ Guidance in applying simple measures to manage lymphedema and hydrocele to prevent progression of lymphedema and debilitating, inflammatory episodes of ADL;
- ➢ Surgery for hydrocele
- ➢ Treatment with antifilarial medicines to destroy any remaining worms and microfilariae by preventive chemotherapy or individual treatment.

Washing

Good hygiene and treatment of entry lesions are important measures for managing lymphedema. The patients should be encouraged to practice skin care and hygiene.

Check skin for

Entry lesions, including very small lesions between the toes that can hardly be seen, (ii) Entry lesions between the toes may cause itching. Scratching can further damage the skin and can provoke an acute attack; tell patients to avoid scratching, (iii) Toenails should be trimmed in such a way that the skin is not injured. Do not try to clean under the nails with sharp objects as these can cause entry lesions.

It is important to check the skin every time the leg is washed because entry lesions allow bacteria to enter the skin and this will cause acute attacks. If entry lesions are found, they should be cleaned carefully.

Wash the leg

(i). Wet the leg with clean water at room temperature. Do not use hot water to wash the leg, (ii). Begin soaping at the highest point of swelling (usually around the knee), (iii). Wash down the leg towards the foot, (iv). Gently clean between all skin folds and between the toes, preferably using a small cloth or cotton swab, and paying particular attention to the entry lesions. Brushes should not be used as they can

damage the skin, (v). Rinse with clean water, (vi). Repeat this careful washing until the rinse water is clean, (vii). Wash the other leg in the same way, even if it looks normal.

Dry the skin

(i). Pat the area lightly with a clean towel. Do not rub hard because this can cause damage to the skin, (ii) Carefully dry between the toes and between skin folds using a small cloth, gauze or cotton swab. Wet areas between the toes, skin folds and entry lesions promote bacterial and fungal growth leading to frequent acute attacks.

PREVENTION AND CURE OF ENTRY LESIONS

Entry lesions are common in patients with lymphedema and are most frequently found between the toes and deep skin folds and around the toenails. Entry lesions, such as wounds, can also be found on the surface of the skin. Both fungi and bacteria can cause entry lesions. Fungal infections frequently damage the skin and create entry lesions, especially between the toes, and may cause itching. The entry lesions allow bacteria to enter the body through the skin and this can cause acute attacks. Fungi and bacteria can cause bad odor.

Fungal infections

Fungal infections are usually white or pink in color and do not leak fluid. Bacterial infections may leak fluid that is thin and clear or thick and colored.

Antifungal and antibacterial creams can be used for local application.

ELEVATION

Elevation **i**s important for patients with lymphedema of the leg. It helps prevent fluid from accumulating in the leg by improving the flow in the elevated position. The knee should be slightly bent and a pillow placed under the knee for support. While sitting, raise the foot as high as is comfortable, preferably as high as the hip. If sitting on the floor, place a small pillow under the knees. If lying down, the foot can be raised by placing a pillow under the mattress.

EXERCISE

Exercise is useful for patients with lymphedema and in general, the more they exercise the better they are. Exercise helps by pumping the fluid and improving drainage. However, patients should not exercise during acute attacks.

WEARING PROPER FOOTWEAR

Proper footwear protects feet from injury.

MANAGEMENT OF ACUTE ATTACKS

The reduction in the frequency of the acute attacks is an indication that the patient's condition is improving. An acute attack is painful. The patient may complain of fever, nausea, headache and soreness of the lymph glands. Most patients can easily care for their acute attack. The patient should rest and elevate the leg comfortably as much as possible at home.

The following simple procedures can alleviate the symptoms:

1. A cloth soaked in water and placed around the leg can relieve pain. The leg can be soaked in bucket of cold water.
2. The leg should be washed with soap and clean water but more gently and carefully.
3. After drying, antiseptic can be applied to the skin and medicated cream.
4. The patient should drink plenty of water
5. Paracetamol can be taken for fever every six hours until the fever lessens.
6. Oral antibiotics can shorten the attack and are recommended.

Conclusion

In this review we have focused on the epidemiology of LF, clinical manifestations, pathogenesis of LF and immune response, current strategy for clinical management and disease management. The creation of GAELF has shown that it is possible to bring different organizations and donations together under one umbrella to co-ordinate and streamline global activities aiming at eliminating LF transmission by 2020. In the still endemic countries, progress has mostly been satisfactory, leading to lower numbers of infected people and to interrupted transmission. Mosquito control is a supplemental strategy supported by WHO. It is used to reduce transmission of lymphatic filariasis and other mosquito- borne infections. Depending on the parasite-vector species, measures such as insecticide-treated nets, indoor residual spraying or personal protection measures may help protect people from infection. Vector control has in select settings contributed to the elimination of lymphatic filariasis in the absence of large-scale preventive chemotherapy.

Key Points

- ✓ Lymphatic filariasis is a neglected mosquito-borne tropical disease which caused by filarial worms, *Wuchereria bancrofti*, *Brugia malayi* or *B. timori* It is endemic in 58 countries, putting 1.2 billion people at risk globally with an estimated 120 million infected
- ✓ Filariasis causes relatively low mortality but has a high incidence of morbidity that has a major social impact, causing heavy economic loss in developing countries
- ✓ It is the second leading cause of permanent or long-term disability with over 40 million infected people suffering from pathological manifestations like lymphoedema, hydrocoele, chyluria and elephantiasis
- ✓ The standard method for diagnosing active infection is by finding the microfilariae via microscopic examination. This may be difficult, as in most parts of the world; microfilariae only circulate in the blood at night. For this reason, the blood has to be collected nocturnally. The blood should be in the form of a thick smear and stained with Giemsa. Testing the blood for filarial antigen has been transformative in enabling the rapid and accurate diagnosis of LF.
- ✓ The clinical manifestations of LF are varied, Traditionally, it has been accepted that people living in an endemic area can be classified into five groups: (1) Endemic Normals; (2) clinically asymptomatic, infected; (3) Acute clinical disease; (4) Chronic pathology and (5) Tropical pulmonary eosinophilia (TPE)
- ✓ While filarial infection does induce expression of immune cells in humans, early interaction of parasites or parasite antigens leads to a predominantly pro-inflammatory response with expression of mainly pro-inflammatory cytokines including TNFα, IL-6 and IL-1β, as well as genes involved in inflammation and adhesion
- ✓ Increase in circulating levels of MMPs and TIMPs is characteristic of the filarial disease process and that that altered ratios of MMP/TIMP are an important underlying factor in the pathogenesis of tissue fibrosis in filarial lymphatic disease
- ✓ Elevated levels of VEGF-A and VEGF-C endothelin-1 have been observed in the serum of filarial-infected individuals
- ✓ Multi-color flow cytometry we have been able to show that the frequency of Th1 cells (CD4+ T cells expressing either IFNγ or IL-2 or TNF-α); Th9 cells (CD4+ T cells expressing IL-9 and IL-10); Th17 cells (CD4+ T cells expressing IL-17) and Th2 cells (CD4+ T cells expressing IL-22) is significantly enhanced in filarial pathology
- ✓ Chronic pathology in lymphatic filariasis is also associated with expanded frequencies of Th9 cells, CD4+ T cells that express both IL-9 and IL-10
- ✓ A basic, recommended package of care can alleviate suffering and prevent further disability among lymphatic filariasis patients.
- ✓ Treatments for lymphatic filariasis differ depending on the geographic location of the endemic area.whereas elsewhere in the world, albendazole is used with diethylcarbamazine. Geo-targeting treatments is part of a larger strategy to eventually eliminate lymphatic filariasis by 2020
- ✓ Lymphatic filariasis can be eliminated by stopping the spread of infection through preventive chemotherapy with single doses of 2 medicines for persons living in areas where the infection is at present.

Acknowledgements:

The authors would like to sincerely thank Dr.Eric Ottesen and Dr. Subash Babu for their critical reading, comments and proofreading. We would like to acknowledge the mentorship and support of late Dr. V. Kumaraswami, who has been instrumental behind this review.

References:

1. Ramaiah KD, and Ottesen EA. Progress and impact of 13 years of the global programme to eliminate lymphatic filariasis on reducing the burden of filarial disease. PLoS Negl Trop Dis. 2014 Nov 20;8(11):e3319
2. **Hooper** PJ, Chu BK, Mikhailov A, Ottesen EA, Bradley M. Assessing progress in reducing the at-risk population after 13 years of the global programme to eliminate **lymphatic filariasis**.PLoS Negl Trop Dis. **2014** Nov 20;8(11):e3333
3. http://www.who.int/mediacentre/factsheets/fs102/en/ as on 08/08/2016
4. AC Dhariwal, PK Srivastava, J Bhattacharjee Journal of the Indian Medical Association, Vol. 113, No. 12, December 2015)
5. Hotez, P.J., Fenwick, A., Savioli, L., Molyneux, D.H., 2009. Rescuing the bottom billion through control of neglected tropical diseases. Lancet 373, 1570–1575.
6. Hotez, P.J., Kamath, A., 2009. Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden. PLoS. Negl. Trop. Dis. 3, e412.
7. WHO, 2011. Metrics Disability-Adjusted Life Year (DALY). Health statis-tics and health information systems, http://www.who.int/healthinfo/global burden disease/metrics daly/en/
8. Kirkwood, B., Smith, P., Marshall, T., Prost, A., 1983. Relationships between mortality, visual acuity and microfilarial load in the area of the Onchocerciasis Control Programme. Trans. R. Soc. Trop. Med. Hyg. 77, 862–868.
9. Little, M.P., Breitling, L.P., Basanez, M.G., Alley, E.S., Boatin, B.A., 2004. Association between microfilarial load and excess mortality in onchocerciasis: an epidemi- ological study. Lancet 363, 1514–1521.
10. Pion, S.D., Kamgno, J., Demanga, N., Boussinesq, M., 2002. Excess mortality associated with blindness in the onchocerciasis focus of the Mbam Valley, Cameroon. Ann.Trop. Med. Parasitol. 96, 181–189.
11. Walker, M., Little, M.P., Wagner, K.S., Soumbey-Alley, E.W., Boatin, B.A., Basanez, M.G., 2012. Density-dependent mortality of the human host in onchocerciasis: relationships between microfilarial load and excess mortality. PLoS Negl. Trop. Dis. 6, e1578
12. Evans, D.B., Gelband, H., Vlassoff, C., 1993. Social and economic factors and the control of lymphatic filariasis: a review. Acta Trop. 53, 1–26.
13. T.F. Schäberle et al.Corallopyronin A – A promising antibiotic for treatment of filariasis International Journal of Medical Microbiology 304 (2014) 72–78
14. Freedman DO, de Almeida Filho PJ, Besh S, Maia e Silva MC, Braga C, Maciel A. Lymphoscintigraphic analysis of lymphatic abnormalities in symptomatic and asymptomatic human filariasis. J Infect Dis. 1994; 170:927–933. [PubMed: 7523538]
15. Freedman DO, de Almeido Filho PJ, Besh S, Maia e Silva MC, Braga C, Maciel A, Furtado AF. Abnormal lymphatic function in presymptomatic bancroftian filariasis. J Infect Dis. 1995; 171:997– 1001. [PubMed: 7706830]
16. Noroes J, Addiss D, Amaral F, Coutinho A, Medeiros Z, Dreyer G. Occurrence of living adult Wuchereria bancrofti in the scrotal area of men with microfilaraemia. Trans R Soc Trop Med Hyg. 1996; 90:55–56. [PubMed: 8730313]
17. Noroes J, Addiss D, Santos A, Medeiros Z, Coutinho A, Dreyer G. Ultrasonographic evidence of abnormal lymphatic vessels in young men with adult Wuchereria bancrofti infection in the scrotal area. J Urol. 1996; 156:409–412. [PubMed: 8683691]
18. Dreyer G, Ottesen EA, Galdino E, Andrade L, Rocha A, Medeiros Z, Moura I, Casimiro I, Beliz F, Coutinho A. Renal abnormalities in microfilaremic patients with Bancroftian filariasis. Am J Trop Med Hyg. 1992; 46:745–751. [PubMed: 1621900]
19. Kabatereine NB, Malecela M, Lado M, Zaramba S, Amiel O, Kolaczinski JH. How to (or not to) integrate vertical programmes for the control of major neglected tropical diseases in sub-Saharan Africa. PLoS Negl Trop Dis 2010; 4(6): e755.
20. Narain JP, Dash AP, Parnell B, Bhattacharya SK, Barua S, Bhatia R, et al. Elimination of neglected tropical diseases in the South- East Asia Region of the World Health Organization. Bull World Health Organ 2010; 88(3): 206-210.
21. Al-Shaham AA, Sood S. Recurrent furunculosis as a cause of isolated penile lymphedema: a case report. J Med Case Reports 2010; 4: 196.
22. Pani SP, Srividya A. Clinical manifestations of bancroftian filariasis with special reference to lymphoedema grading. Indian J Med Res. 1995; 102:114–118. [PubMed: 8543349]
23. Dreyer G, Medeiros Z, Netto MJ, Leal NC, de Castro LG, Piessens WF. Acute attacks in the extremities of persons living in an area endemic for bancroftian filariasis: differentiation of two syndromes. Trans R Soc Trop Med Hyg. 1999; 93:413–417. [PubMed: 10674092]
24. Okon OE, Iboh CI, Opara KN. Bancroftian filariasis among the Mbembe people of Cross River state, Nigeria. J Vector Borne Dis 2010; 47(2): 91-96.

25. Partono F. The spectrum of disease in lymphatic filariasis. Ciba Found Symp. 1987; 127:15–31. [PubMed: 3297555]
26. Okon OE, Iboh CI, Opara KN. Bancroftian filariasis among the Mbembe people of Cross River state, Nigeria. J Vector Borne Dis 2010; 47(2): 91-96.
27. Edeson JF.The epidemiology and treatment of infection due to Brugia malayi. Bull World Health Organ 1962; 27(4-5): 529- 541.
28. Ong RK, Doyle RL. Tropical pulmonary eosinophilia. Chest. 1998; 113:1673–1679. [PubMed: 9631810]
29. Ottesen EA, Nutman TB. Tropical pulmonary eosinophilia. Annu Rev Med. 1992; 43:417–424. [PubMed: 1580599]
30. McCarthy J. Diagnosis of lymphatic filarial infection. In: Nutman TB. Lymphatic filariasis. London: Imperial College Press; 2000,p. 127-141
31. Weil G, Lammie PJ, Weiss N. The ICT filariasis test: A rapid format antigen test for diagnosis of bancrofitian flariasis. Parasitol Today 1997; 13: 401-404.
32. Amaral F, Dreyer G, Figueredo-Silva J. Live adult worms detected by ultrasonography in human bancroftian filariasis. Am J Trop Med Hyg 1994; 50: 735-757.
33. Anitha, K Shenoy RK. Treatment of lymphatic filariasis. Curr Trends Continuing Medical Education 2001: 67(2): 60-65.
34. Stingl P, Ross M, Gibson DW *et al.* A diagnostic 'patch test' for onchocerciasis using topical diethylcarbamazine. *Trans. R. Soc. Trop. Med. Hyg.* 78(2), 254–258 (1984).
35. Dreyer G, Noroes J, Figueredo-Silva J, Piessens WF. Pathogenesis of lymphatic disease in bancroftian filariasis: a clinical perspective. Parasitol Today. 2000; 16:544–548. [PubMed: 11121854]
36. Dreyer G, Noroes J, Addiss D, Santos A, Medeiros Z, Figueredo-Silva J. Bancroftian filariasis in a paediatric population: an ultrasonographic study. Trans R Soc Trop Med Hyg. 1999; 93:633–636. [PubMed: 10717753]
37. Amaral F, Dreyer G, Figueredo-Silva J, Noroes J, Cavalcanti A, Samico SC, Santos A, Coutinho A. Live adult worms detected by ultrasonography in human Bancroftian filariasis. Am J Trop Med Hyg. 1994; 50:753–757. [PubMed: 8024070]
38. Dreyer G, Amaral F, Noroes J, Medeiros Z. Ultrasonographic evidence for stability of adult worm location in bancroftian filariasis. Trans R Soc Trop Med Hyg. 1994; 88:558. [PubMed: 7992338]
39. Figueredo-Silva J, Noroes J, Cedenho A, Dreyer G. The histopathology of bancroftian filariasis revisited: the role of the adult worm in the lymphatic-vessel disease. Ann Trop Med Parasitol. 2002; 96:531–541. [PubMed: 12396316]
40. Connor DH, Palmieri JR, Gibson DW. Pathogenesis of lymphatic filariasis in man. Z Parasitenkd. 1986; 72:13–28. [PubMed: 3515791]
41. von Lichtenberg F. The Wellcome Trust lecture. Inflammatory responses to filarial connective tissue parasites. Parasitology. 1987; 94(Suppl):S101–S122. [PubMed: 3295688]
42. Olszewski WL, Jamal S, Manokaran G, Pani S, Kumaraswami V, Kubicka U, Lukomska B, Dworczynski A, Swoboda E, Meisel-Mikolajczyk F. Bacteriologic studies of skin, tissue fluid, lymph, and lymph nodes in patients with filarial lymphedema. Am J Trop Med Hyg. 1997; 57:7– 15. [PubMed: 9242310].
43. Shenoy RK, Kumaraswami V, Suma TK, Rajan K, Radhakuttyamma G. A double-blind, placebo- controlled study of the efficacy of oral penicillin, diethylcarbamazine or local treatment of the affected limb in preventing acute adenolymphangitis in lymphoedema caused by brugian filariasis. Ann Trop Med Parasitol. 1999; 93:367–377. [PubMed: 10656038].
44. Allen JE and Maizels RM. Diversity and dialogue in immunity to helminths. *Nat. Rev.Immunol* 2011; 11:375–388.
45. McSorley HJ, Maizels RM. Helminth infections and host immune regulation. *Clin. Microbiol. Rev* 2012; 25:585–608.
46. Lal RB, Dhawan RR, Ramzy RM, Farris RM, Gad AA. C-reactive protein in patients with lymphatic filariasis: increased expression on lymphocytes in chronic lymphatic obstruction. J Clin Immunol. 1991; 11:46–53. [PubMed: 2022721]
47. Das BK, Sahoo PK, Ravindran B. A role for tumour necrosis factor-alpha in acute lymphatic filariasis. Parasite Immunol. 1996; 18:421–424. [PubMed: 9229396]
48. Satapathy AK, Sartono E, Sahoo PK, Dentener MA, Michael E, Yazdanbakhsh M, Ravindran B. Human bancroftian filariasis: immunological markers of morbidity and infection. Microbes Infect. 2006; 8:2414–2423. [PubMed: 16839794]
49. el-Sharkawy IM, Haseeb AN, Saleh WA. Serum levels of endothelin-1 (ET-1), interleukin-2 (IL-2) and amino-terminal propeptide type III procollagen (PIII NP) in patients with acute and chronic filariasis. J Egypt Soc Parasitol. 2001; 31:169–176. [PubMed: 12557940]
50. Babu S, Blauvelt CP, Kumaraswami V, Nutman TB. Chemokine receptors of T cells and of B cells in lymphatic filarial infection: a role for CCR9 in pathogenesis. J Infect Dis. 2005; 191:1018– 1026. [PubMed: 15717282]
51. Bennuru S, Nutman TB: Lymphangiogenesis and lymphatic remodeling induced by filarial parasites: implications for pathogenesis. PLoS Pathog. Dec 2009;5:e1000688.
52. Anuradha R: Altered circulating levels of matrix metallproteinases and inhibitors associated with elevated Type 2 cytokines in lymphatic filarial disease. PLoS Negl Trop Dis. 2012.
53. Pfarr KM, Debrah AY, Specht S, Hoerauf A: Filariasis and lymphoedema. Parasite Immunol. Nov 2009;31:664–672.
54. Debrah AY, Mand S, Specht S, et al.: Doxycycline reduces plasma VEGF-C/sVEGFR-3 and improves

pathology in lymphatic filariasis. PLoS Pathog. Sep 2006;2:e92.
55. Bennuru S, Maldarelli G, Kumaraswami V, Klion AD, Nutman TB: Elevated levels of plasma angiogenic factors are associated with human lymphatic filarial infections. Am JTrop Med Hyg. Oct 2010;83:884–890.
56. Brenchley JM, Douek DC: Microbial translocation across the GI tract. Annu Rev Immunol. 2012;30:149 173.
57. Anuradha R: Circulating microbial products and acute phase proteins as markers of pathogenesis in lymphatic filarial disease. PLoS Pathog. 2012.
58. Anuradha R, George PJ, Kumaran P, Nutman TB, Babu S. "Interleukin-10- and transforming growth factor β-independent regulation of $CD8^+$ T cells expressing type 1 and type 2 cytokines in human lymphatic filariasis." Clin Vaccine Immunol. 2014 Dec;21(12):1620-7. doi: 10.1128/CVI.00598-14
59. Anuradha R, George PJ, Hanna LE, Chandrasekaran V, Kumaran P, Nutman TB, Babu S. "IL-4-, TGF-β-, and IL-1-dependent expansion of parasite antigen-specific Th9 cells is associated with clinical pathology in human lymphatic filariasis." J Immunol. 2013 Sep 1;191(5):2466-73. doi: 10.4049/jimmunol.1300911
60. Anuradha R, George PJ, Hanna LE, Chandrasekaran V, Kumaran PP, Nutman TB, Babu S. "Parasite-antigen driven expansion of IL-5(-) and IL-5(+) Th2 human subpopulations in lymphatic filariasis and their differential dependence on IL-10 and TGFβ." PLoS Negl Trop Dis. 2014 Jan 30;8(1):e2658. doi: 10.1371/journal.pntd.0002658
61. Anuradha R, George PJ, Chandrasekaran V, Kumaran PP, Nutman TB, Babu S. "Interleukin 1 (IL-1)- and IL-23-mediated expansion of filarial antigen-specific Th17 and Th22 cells in filarial lymphedema." Clin Vaccine Immunol. 2014 Jul;21(7):960-5. doi: 10.1128/CVI.00257-14
62. Babu S, Kumaraswami V, Nutman TB. Alternatively Activated and Immunoregulatory Monocytes in Human Filarial Infections. J Infect Dis. 2009; 199:1827–1837. [PubMed: 19456233]
63. Sabesan S, Palaniyandi M, Das PK, Michael E. Mapping of lymphatic filariasis in India. Ann Trop Med Parasitol 2000;94:591-606.
64. World Health Organization (2010) WHO global programme to eliminate lymphatic filariasis progress report for 2000–2009 and strategic plan 2010–2020. Geneva: World Health Organization.
65. Dreyer G, Addiss D, Dreyer P, Noroes J (2002) Basic lymphedema management: treatment and prevetion of problems associated with lymphatic filariasis. Hollis, NH: Hollis Publishing.
66. www.thelancet.com/infection India tackles lymphatic filariasis Sanjeet Bagcchi reports Vol 15 April 2015
67. Manokaran G. Lymph node-venous microvascular reconstructive surgery:Lymphedema Filariasis. Lymphedema: A concise compendium of theory and Practice:(c)Springer-Verlag London Limited. 2011. Editors, Lee B. B , Begran John and Stanley G.: 365-368.
68. Sahare KN, Anandharaman V, Meshram VG, Meshram SU, Gajalakshmi D,Goswami K, et al. In vitro effect of four herbal plants on the motility of Brugia malayi microfilariae. Indian J Med Res 2008; 127: 467-471.
69. Wabo Pone J, Bilong Bilong CF, Mpoame M. In vitro nematicidal activity of extracts of Canthium mannii (Rubiaceae), on different life-cycle stages of Heligmosomoides polygyrus (Nematoda, Heligmosomatidae). J Helminthol 2009; 1-10.
70. Jain SP, Singh J. Traditional medicinal practice among the tribal people of raigarh (Chattishgarh), India. Ind J Nat Product Resources 2010; 1: 109-115.
71. Misra N, Sharma M, Raj K, Dangi A, Srivastava S, Misra-Bhattacharya S. Chemical constituents and antifilarial activity of *Lantana camara* against human lymphatic filariid *Brugia malayi* and rodent filariid *Acanthocheilonema viteae* maintained in rodent hosts. *Parasitol. Res*. 100(3), 439–448 (2007).
72. Govt. of India, Ministry of Health and Family Welfare: National vector borne diseases control programme; Problems and elimination of lymphatic filariasis in India. 2005.Available from: http://www.namp.gov.in/filariasis.html . [cited on 2010 Jan 12].
73. Pani SP, Subramanyam Reddy G, Das LK, Vanamail P, Hoti SL, Ramesh J, et al. Tolerability and efficacy of single dose albendazole, diethylcarbamazine citrate (DEC) or co-administration of albendazole with DEC in the clearance of Wuchereria bancrofti in asymptomatic microfilaraemic volunteers in Pondicherry, South India: A hospital-based study. Filaria J 2002;1:1.
74. Shenoy RK, Dalia S, John A, Suma TK, Kumaraswami V. Treatment of the microfilaraemia of asymptomatic brugian filariasis with single doses of ivermectin, diethylcarbamazine or albendazole, in various combinations. Ann Trop Med Parasitol 1999;93:643
75. Shenoy RK, Suma TK, John A, Arun SR, Kumaraswami V, Fleckenstein LL, et al. The pharmacokinetics, safety and tolerability of the co-administration of diethylcarbamazine and albendazole. Ann Trop Med Parasitol 2002;96:603-14. Sunish IP, Rajendran R, Mani TR, Dash AP, Tyagi BK. Evidence for the use of albendazole for the elimination of lymphatic filariasis. Lancet Infect Dis 2006;6:125-6.
76. Sabesan S. Albendazole for mass drug administration to eliminate lymphatic filariasis. Lancet Infect Dis 2006;6:684-5.

CAMBRIDGE UNIVERSITY PRESS

Toll against helminth parasites

Journal:	*Parasitology*
Manuscript ID	PAR-2016-0311
Manuscript Type:	Review - Standard
Date Submitted by the Author:	25-Aug-2016
Complete List of Authors:	Rajasekaran, Sivaprakasam; National Institute for Resaerch in Tuberculosis, Department of Immunology Anuradha, Rajamanickam; ICER-NIH-NIRT, Department of Immunology Bethunaickan, Ramalingam; National Institute for Resaerch in Tuberculosis, Department of Immunology
Key Words:	Helminth infection, TLR, Immune response to helminthes

Toll against helminth parasites

Sivaprakasam Rajasekaran[1], Rajamanickam Anuradha[2] and Ramalingam Bethunaickan[1 #]

1 Department of Immunology, National Institute for Research in Tuberculosis, Chennai –India

2 International Center for Excellence in Research - National Institute of Health – National Institute for Research in Tuberculosis, Chennai, India.

#Address Correspondence to:

Dr. Ramalingam Bethunaickan, Ph.D.,
Scientist D & DBT Ramalingaswami Fellow,
Department of Immunology,
National Institute for Research in Tuberculosis (ICMR),
No.1. Mayor Satyamoorthy Road,
Chetpet, Chennai- 600 031.

Telephone : +91 44 2836 9761
Fax : +91 44 2836 2525
E-mail address: bramalingam@gmail.com

Key words: Helminth infection, TLR, Immune response to helminthes.

Abstract

Helminth (worm) infections are major public health problems that have important socioeconomic consequences for the more than 2 billion infected individuals. The hallmark of helminth infection is their Chronicity can lead to anemia (in hookworm infection), cirrhosis (schistosomiasis), and elephantiasis (lymphatic filariasis). Helminth parasites have developed mechanisms to evade host responses allowing them to survive in hostile environments such as the gastrointestinal tract, the lymphatics, and the bloodstream. The maintenance of the asymptomatic state is now recognized as reflecting an immunoregulatory environment, which may be promoted by parasites, and involves multiple levels of host regulatory cells and cytokines; a breakdown of this regulation is observed in pathological disease. Although there have been many studies examining innate immune responses (including TLR expression and function) in response to intracellular pathogens, fewer have examined the interaction of the multicellular helminth parasites and innate immune system. Understanding and exploiting the interactions between these parasites and the host regulatory network are therefore likely to highlight new strategies to control both infectious and immunological diseases. This review will focus on systemic helminth parasitic infections and helminth-derived products which elicit both communal and unique host responses and the regulation of TLRs that may contribute to infection outcome.

Background:

Parasitic helminths, or worms, comprise a diverse group of metazoan organisms that infects over one billions of people in sub- Saharan Africa, Asia and the Americas

are infected with one or more helminth species. (1-2). Helminth infections are a major public health problem resulting in many physical disabilities and having important socio-economic impact. Infections by these pathogens are generally not fatal; they are associated with high rates of morbidity, with chronic infection often leading to physical disabilities, anemia and malnourishment (3). The overall prevalence of helminthiases is so high, relatively low frequencies of severe disease neverthe-less equate to large numbers of people experiencing infection-associated morbidity. Due to the control of insect vector populations, the safe disposal of human excrement, and the availability of efficacious drugs, helminth parasites have been largely eradicated as a public health concern in developed countries, unfortunately, however, in developing countries, where these types of control measures are often not yet practical, helminths remain a significant biomedical problem.

Lifecycle:

Parasitic helminths have evolved mechanisms to overcome and evade host immune responses to thrive in immune-exposed locations such as lymphatics, bloodstream, and gastrointestinal tract. (4). Many of the parasitic worms have complex multistage life cycles that involve several hosts. Within their mammalian hosts they often undergo extensive growth and differentiation with the ultimate goal of producing stages intended for transmission to the next intermediate host. Usually, the life stage responsible for infecting the mammalian host is the larva, and the larva must migrate within the host to its appropriate niche where it can grow and reproduce. Since the offspring are intended for transmission to another animal, they must necessarily be capable in some way of entering a site from which they can leave the host. How all this is accomplished varies from one helminth to another (5). The chronicity, disability, social impact, and overall burden of these worm infections have

led to much research on the immune responses and of pathogenesis of helminth infections. Modulation of the host immune response involves a variety of strategies, including induction of regulatory networks and dysregulation of innate and adaptive immune responses (6); studies in endemic areas suggest both innate and adaptive immune systems play a role in host defense

Helminth parasites have evolved immune evasion strategies necessary for their continued transmission. This immune evasion is achieved at the expense of both antigen-presenting cells (APCs) and T cells .The interactions of helminth parasites with APCs are known to involve Pattern-recognition receptors that form a key component of microbial detection and host defense and are important in the initiation of host immune responses (6). TLRs are involved in recognition of a wide spectrum of pathogens by binding to pathogen-associated molecular patterns (7). TLR control multiple APC functions and activate signals critically involved in the initiation of adaptive immune responses (8). In recent years, it has been shown that APCs recognize these PAMPS through TLRs and NLRs leading to signaling (9,10) through pathways that induce production of inflammatory cytokines.

TLRs Ligand and Structure:

Thirteen TLRs have been currently identified, TLR1 to TLR13, of which TLR1 to TLR9 are conserved both in human and mice. TLR10 is not functional in mice while TLR11, TLR12 and TLR13 are absent from human genome (11) TLRs are type-1 transmembrane proteins that are pattern recognition receptors (PRRs) that function as sensors for innate immune responses that, in turn, direct the responses of the adaptive immune system (12). They are expressed in different combinations on many cells of the immune system, at the cell surface. Extracellular TLR domains have reiterated leucine- rich modules bearing pathogen-associated molecular patterns

(PAMPs) able to recognize a wide range of microbial products, thus providing a link between innate and adaptive immunity (13, 14)

TLR Expression:

In mammals, specific combinations of TLRs are expressed on immune cells such as DCs, macrophages, mast cells, and to a lesser degree in fibroblasts, epithelial cells, endothelial cells, and also in T and B cells. The microbial products comprising PAMPs that bind to TLR can be lipids, lipo-peptides, proteins, or nucleic acids. Initially, Endogenous ligands such as self-molecules from apoptotic cells can also trigger inflammation. TLRs can bind to endogenous heat-shock proteins, extra-cellular matrix fragments, fibrinogen, and self-RNA and DNA (15). Endogenous RNA and DNA are able to activate TLR7 and TLR9 if they enter endosomal compartment, inducing production of pro-inflammatory cytokines by plasmocytoid DCs (16). Protozoan parasites such as Trypanosome spp., E.histolytica have been shown to inhibit immune response, particularly by down regulating TLR-2 expression (17). Monocyte derived DC to live MF of BmA significantly down regulate the mRNA expression of TLR3, TLR4, TLR5 and TLR7 (18). Filarial infected individuals have shown decreased expression of TLR1, TLR2, TLR4, and TLR9 on mRNA expression and protein in B cells (19). T cells express many of the TLRs at the mRNA gene level and therefore may play an additional role in TLR signaling. T cells from patients with lymphatic filariasis, in the presence of B cells and monocytes, expressed lower levels of TLR1, 2, and 4 (20).

Immune response to Helminths:

A diverse range of parasites have adopted humans as their definite hosts and reside in various organs and lymphatics, the invoked immune responses by the host are surprisingly stereotypical (21-23). Helminth parasites are known to release a wide

variety of enzymatically active products that are thought to play an important role in establishing and maintaining infection, by contributing to the degradation of soluble anti-parasitic molecules or through impairment of innate immune cells (24)

These terminate in the production of various Th2 associated cytokines (IL-5, IL-13, IL-4) and immunoglobulins (IgE) from effector cells which all stem from a discordance of cellular responses such as mast cell and eosinophil mobilization. The absolute requirement for type 2 immunity has been demonstrated in various infection systems but this response rarely results in expulsion of the parasite (25). Pre-established infection with the helminth Schistosoma mansoni, or pre-treatment of mice with S. mansoni ova significantly attenuates clinical course, reduces incidence and delays onset of EAE in mice, and is associated with decreased IFN-γ, TNF-α, IL-12 and nitric oxide production by splenocytes, as well as increased production of IL-10 and TGF-b (26, 27). The filarial endosymbiont Wolbachia is known to elicit immune responses through TLR2 and TLR4 and is known to be the major mediator of inflammatory responses in lymphatic filariasis and onchocerciasis (28, 29). Helminths can elicit severe immunopathology, the majority of infections remain asymptomatic, that is, the host tolerates the worm and it is this immunomodulatory capacity of the worm that currently fascinates many immunologists (30).

Immune modulation by Helminths:

TLRs trigger an intracellular signaling cascade through TIR (31) and through the recruitment of adaptor molecules, such as TICAM-1, MyD88 and TRIF, and TRAM (31, 32). These adaptor molecules act independently, or in combination, based on the TLRs and trigger NF- kB, c-Jun-N-terminal kinase (JNK), mitogen-activated protein kinases (MAPK), p38, extracellular signal-regulated kinase (ERK) and NF- B leading to the transcription of inflammatory and immunomodulatory genes including

co-stimulatory molecules, cytokines and chemokines (34, 35). Several studies have demonstrated that ongoing infections in the absence of certain TLR, deviates adaptive responses, which exacerbates the immunopathology of the host. This is of particular interest when studying parasitic infections, such as helminths since they utilize such immune-regulation for their own survival (37, 38). **T**he potential role of TLR in mediating interactions between helminth parasites and the host immune system has recently been described. TLR-triggering of DC promotes pro-inflammatory/Th1 environments, which would theoretically coincide with the typically induced Th1 responses, observed in the acute phase of helminth infections (21-23, 25)**.** Many helminth antigen preparations contain a large mixture of proteins, glycoproteins, and glycolipids and whereas some have been shown to modulate various TLR others have been shown to activate other PRR families. The calreticulin protein, isolated from the H.polygyrus can induce IL-4 production through activation of the class A scavenger receptor (36). Helminth antigens are more prominent for their dispassionate behavior on DC activation; their failure to induce conventional pro-inflammatory activation and maturation. *A. viteae ES-62* product contains glycoprotein, which triggers TLR4 in turn induces an anti-inflammatory and Th2 induces APC phenotype (42, 43, 45). Soluble egg antigen (SEA), derived from S. mansoni eggs, does not elicit TLR-triggered responses when co-cultured with innate cells as such but actually dampens pro-inflammatory cytokine release elicited upon co-culture with LPS (39, 41). Schistosomes have been shown to induce immune responses through TLR2 promotes the differentiation of DCs that induce regulatory T cells, which secrete the anti-inflammatory cytokine IL-10 (45). TLR2 is a receptor that plays an important role in filarial infection, the filarial endosymbiont Wolbachia is known to elicit immune responses through TLR2 and TLR4 and is known to be the major mediator of

inflammatory responses in lymphatic filariasis and onchocerciasis (46, 47). Filarial parasites harbor a *Wolbachia* endosymbiont that can interact with the innate immune system through TLR2 and TLR4 (28). Individuals with filarial infection have diminished expression of APC- and T cell- specific-TLR1, 2, 4, and 9 as well as decreased pro-inflammatory cytokine responses to TLR2, 4, and 9 ligands (19-20).

TLR signaling

Down-regulation of TLR-mediated immune responses through dampening TLR-mediated cell signaling or through diminished TLR expression seems to be an important immune evasion mechanism in some bacterial pathogens (48), a process that may be applicable to helminth parasites (49). The activation of TLRs by helminth-derived molecules leads to different down- stream activation of kinases involved in intracellular signaling compared to TLR activation by Th1 stimuli. The binding of TLRs triggers a series of signaling events that lead to the activation of the nuclear factor-kB (NF-kB) pathway and, as a result, induction of inflammatory responses. TLRs have a common conserved domain (TIR) that is located intracellularly. Once this Toll/ IL-1R (TIR) domain is activated, it initiates a signal through five different adaptor molecules that eventually leads to activation of the NF-kB-dependent pathway along with the interferon regulatory factor (IRF) pathway (51). After interaction with a specific ligand, the TLR recruits an adaptor protein to its TIR domain. The first discovered adaptor molecule was myeloid differentiation primary response gene 88 (MyD88), a molecule involved in the signaling pathway for all TLRs except TLR3. MyD88 contains a death domain at its N-terminus and a TIR domain at its C-terminus. It often couples with MyD88 adaptor-like (MAL) protein for certain TLR signaling. Another adaptor protein, TIR-related adaptor protein

inducing interferon (TRIF), is the sole adaptor molecule for TLR3 that signals through a MyD88-independent pathway. TRIF interacts with adaptor molecule TRIF-related adaptor molecule (TRAM) for signaling through TLR4, which also can signal through the MAL/MyD88 adaptor protein complex. The TLR4 ligand LPS has been found to strongly activate MAP-kinases p38, JNK and ERK, the molecule LNFPIII, acts via TLR4, phosphorylates only ERK. The MyD88-independent pathway, TLR4 can induce activation of IRF3 and induce production of IFN through the TRAM/TRIF complex (52-54). NF- kB is a major regulator of gene transcription made up of five subunits: p50, p65, p52, RelB, and c-Rel (55). Two of these subunits dimerize to allow translocation into the nucleus, and NF-kB binds to DNA. Once in the nucleus, NF-kB regulates the production of more than 150 genes coding for cytokines, Ag receptors, apoptosis, and host defense. Both sensory and effector functions of TLRs are involved during immune response to pathogens. The production of pro-inflammatory cytokines and increased APC co-stimulatory potential are perhaps the immediate response of the host to pathogens by the host via TLR recognition of that particular pathogen (56) One of the major signaling cascades triggered as a result of TLRs engagement is the MAPK pathway. Differential activation of MAPK p38 and extracellular-signal-regulated kinase (ERK) in DCs has been associated with varying levels of cell maturation and cytokine production. p38 is thought to be important in DC- maturation, and pro-inflammatory T cell responses, whereas ERK activation has more often been associated with anti-inflammatory and Th2 responses (57, 58).In SEA model, during infections, absence of TLR2 leads to enhanced the disease severity, Th1 and diminished Th2 responses (59).SEA, Giardia extracts are down regulated the expression of LPS induced co-stimulatory molecules on DCs in a MYD88-independent manner (58, 60). SEA and TLR2 agonists modulate intracellular

pathways resulting in suppression of IL-12, IL-6, IL-1β, and TNF-α DC production, and activation of TGF-β, as well as IL-10 production by both DCs and B cells through a MyD88-dependent pathway (58). ES-62 a glycoprotein from the rodent nematode A.vitae inhibited both B and T cell activation through the MyD88-dependent TLR4 pathway (61). The p38 MAPK and ERK1/2 pathways, as well as NF-kB activation, play an important role in mediating the angiogenic growth factor response to TLR ligands in CP individuals (62). Down regulation of TLR on antigen-presenting cells and T cells has been shown to be a possible mechanism by which deleterious pathology in clinically asymptomatic filarial infections can be circumvented (63). Many studies on TLR signaling in response to intracellular pathogens (including the parasitic protozoa) (64-66) the evidence that filarial parasites elicit immune response through TLRs [67]. Wolbachia extracts derived from a mosquito cell line induced similar LPS-dependent response in murine macrophages, perhaps through TLR4. The major surface protein of Wolbachia (wsp) in filarial nematodes can indeed elicit immune response in human embryonic kidney 293 (HEK293) cell line through both TLR2 and TLR4 (68) wsp induced an inflammatory response measured by pro-inflammatory cytokines in murine macrophages and DCs again through a TLR2- and TLR4-dependent mechanism, as mice deficient in either of these TLRs failed to elicit the same response. (69) Using human TLR- transfected HEK cell line as well as murine macrophages from TLR and adaptor molecule gene knockout to show that the inflammatory response to Wolbachia is mediated primarily by engagement of TLR2 and TLR6 and is indeed dependent on MyD88 and the TIR domain-containing adaptor protein (TIRAP)/MAL.

Activation of Toll-like receptors by helminth derived molecules:

Helminth parasites are known to release a wide variety of enzymatically active products that are thought to play an important role in establishing and maintaining infection, by contributing to the degradation of soluble anti-parasitic molecules or through impairment of innate immune cells (70) Down regulation of an Ag-specific T cell proliferative response is a hallmark of several different parasitic infections (71-73) and may reflect a mechanism by which parasite survival is promoted in the host. The rodent filarial nematode A.viteae, the phosphorylcholine-containing glycoprotein ES-62 was found to inhibit the activation of B and T lymphocytes through TLR recognition (74). Live Schistosoma larvae of different maturation stages or soluble preparations from whole larvae were used to stimulate cytokine production by thioglycollate-elicited macrophages, the parasite-derived molecules released from the schistosome larvae were shown to partly act through TLR4, MyD88-dependent pathway (75). Omega-1 a glycoprotein derived from schistosoma eggs specifically primes DCs to drive polarized Th2 responses (70). A. vitae which exerts several immunomodulatory effects including inhibition of B- and T-lymphocyte proliferation, inhibition of IFN-γ, IL-12, and IL-17 secretion, and inhibition of maturation of naive DCs priming T cells (76). Schistosoma product, Sm16 activates the production of IL-1 receptor antagonists from human keratinocytes, inducing secretion IL-10 and down-regulating ICAM- 1 expression. Lacto-N-fucopentose III (LNFP III) contained in Schistosoma eggs is an oligosaccharide that induces IL-10 producing B1 cells in mice (77). Schistosomal and ascaris derived lipids were found to signal via TLR2 (78)

Conclusion:

In this review we have described multiple helminth-derived products that elicit both communal and unique host responses. Understanding the mechanisms that mediate the effects that helminths have on the immune system will provide us with

information that can be manipulated to prevent inflammatory diseases or immunopathology. For example, characterizing how helminth derived molecules interact with TLRs to stimulate an anti-inflammatory response. This molecule is involved in the induction of Th2 and its absence is associated with increased autoimmune disease. Some of these mechanisms are TLR dependent either at the innate or adaptive side and such immunomodulation appears to benefit the worm in its long-term relationship with the host. Unraveling those molecules will hopefully provide new strategies in preventing allergy and auto immunity diseases.

Acknowledgements:

The authors would like to sincerely thank Dr. Subash Babu, Scientific Director, NIH-NIRT-ICER, for his critical reading, comments and proofreading. We would like to acknowledge the mentorship and support of late Dr. V. Kumaraswami, who has been instrumental behind this review. RB's work is supported by DBT Ramalingaswami Fellowship, Ministry of Science and Technology, India.

REFERENCES

1. Research Priorities for helminth infections Technical Report of the TDR Disease Reference group on helminth infection 2012
2. ***Colley, D. G., P. T. LoVerde, and L. Savioli.*** *(2001). (Medical helminthology in the 21st century. Science 293:1437–1438.)*
3. **WHO**. World Health Report. World Health Organization; Geneva, Switzerland: 1999
4. **Mencl F, Birkle M, Blanda M, Gerson LW**. EMTs' knowledge regarding transmission of infectious disease. Prehosp Emerg Care. 2000; 4:57–61
5. Immunology of Parasitic Helminth Infections *Andrew S. MacDonald, Maria Ilma Araujo and Edward J. Pearce Infect. Immun. 2002, 70(2):427. DOI: 10.1128/IAI.70.2.427-433.2002*
6. ***Maizels RM, Yazdanbakhsh M*** *(2003) Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 3: 733–744*
7. **Barton GM, Medzhitov R**. (2002) Toll like receptors and their ligands. *Curr Top Microbiol Immunol*; 270:81-92.
8. **Iwasaki, A., R. Medzhitov**. 2004. Toll-like receptor control of the adaptive immune responses. *Nat. Immunol.* **5**: 987-995.
9. **Blander JM, Medzhitov R**. Regulation of phagosome maturation by signals from Toll-like receptors. Science. 2004; 304:1014–8.
10. **Mariathasan S, Monack DM**. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. Nat Rev Immunol. 2007; 7:31–40. [PubMed: 17186029]
11. **T. Kawai and S. Akira**, 2010 "The role of pattern-recognition re- ceptors in innate immunity: update on toll-like receptors," *Nature Immunology*, vol. 11, no. 5, pp. 373–384
12. **Medzhitov R, Preston-Hurlburt P, Janeway CA Jr**. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature*. 1997; 388:394–7. [PubMed: 9237759]
13. **Akira, S**. (2003). Mammalian Toll-like receptors. Curr. Opin. Immunol. 15, 5–11.;

14. **Kawai, T., and Akira, S.** (2005). Pathogen recognition with Toll-like receptors. *Curr. Opin. Immunol*. 17, 338–344.

15. **Yu, L., Wang, L., and Chen, S.** (2010). Endogenous Toll-like receptor ligands and their biological significance. *J. Cell Mol. Med*. 14, 2592–2603

16. **Chi, H., and Flavell, R. A.** (2008). Innate recognition of non-self nucleic acid. Genome Biol. 9, 211.1–211.6

17. **Maldonado C, Trejo W, Ramirez A, Carrera M, Sanchez J, Lopez-Macias C,** et al. Lipophosphopeptidoglycan of Entamoeba histolytica induces an antiinflammatory innate immune response and downregulation of Toll-like receptor 2 (TLR-2) gene expression in human monocytes. *Arch Med Res*. 2000; 31:S71–3. [PubMed: 11070229]

18. **Semnani RT, Venugopal PG, Leifer CA, Mostbock S, Sabzevari H, Nutman TB**. Inhibition of TLR3 and TLR4 function and expression in human dendritic cells by helminth parasites. *Blood*. 2008; 112:1290–8. [PubMed: 18541719]

19. **Babu S, Blauvelt CP, Kumaraswami V, Nutman TB**. Diminished expression and function of TLR in lymphatic filariasis: a novel mechanism of immune dysregulation. *J Immunol*. 2005; 175:1170– 6. [PubMed: 16002719]

20. **Babu S, Blauvelt CP, Kumaraswami V, Nutman TB**. Diminished T cell TLR expression and function modulates the immune response in human filarial infection. *J Immunol*. 2006; 176:3885– 9. [PubMed: 16547219]

21. **Maizels, R. M., and Yazdanbakhsh, M**. (2003). Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat. Rev. Immunol*. 3, 733–744.

22. **Maizels, R. M., Balic, A., Gomez- Escobar, N., Nair, M., Taylor, M. D., and Allen, J. E.** (2004). Helminth parasites – masters of regulation. *Immunol. Rev*. 201, 89–116.

23. **Anthony, R. M., Rutitzky, L. I., Urban, J. F. Jr., Stadecker, M. J., and Gause, W. C.** (2007). Protective immune mech- anisms in helminth infection. *Nat. Rev. Immunol*. 7, 975–987.

24. **Everts, B., Smits, H. H., Hokke, C. H., and Yazdanbaksh, M.** (2010). Helminths and dendritic cells: sens- ing and regulating via pattern recog- nition receptors, Th2 and Treg responses. *Eur. J. Immunol*. 40, 1525–1537.

25. **Allen, J. E., and Maizels, R. M.** (2011). Diversity and dialogue in immunity to helminths. *Nat. Rev. Immunol*. 11, 375–388.

26. **La Flamme, A. C., Ruddenklau, K., and Bäckström, B. T.** (2003). Schistosomiasis decreases central nervous inflammation and alters the progression of experimental.. *Infect. Immun*. 71, 4996–5004.

27. **Sewell, D., Qing, Z., Reinke, E., Elliot, D., Weinstock, J., Sandor, M., and Fabry, Z.** (2003). Immunomodualtion of experimen- tal autoimmune encephalomyelitis by helminth ova immunization. **Int. Immunol**. 15, 59–69.

28. **Brattig, N. W., C. Bazzocchi, C. J. Kirschning, N. Reiling, D. W. Buttner, F. Ceciliani, F. Geisinger, H. Hochrein, M. Ernst, H. Wagner, et al**. 2004. The major surface protein of Wolbachia endosymbionts in filarial nematodes elicits immune re- sponses through TLR2 and TLR4. *J. Immunol*. 173: 437– 445.

29. **Taylor,M.J.,H.F.Cross,andK.Bilo**.(2000).Inflammatoryresponsesinduce dbythe filarial nematode Brugia malayi are mediated by lipopolysaccharide-like activity from endosymbiotic Wolbachia bacteria. *J. Exp. Med.* 191: 1429 –1436.

30. **Moreau, E., and Chauvin, A**. (2010). Immunity against helminths: inter- actions with the host and the intercurrent infections. *J. Biomed. Biotechnol.* 2010, 428593.

31. **Mustelin T**: Restless T cells sniff and go. *Science* 2006, 313:1902-1903.

32. **Nagpal K, Plantinga TS, Sirois CM, Monks BG, Latz E, Netea MG, Golenbock DT**: Natural loss-of-function mutation of myeloid differentiation protein 88 disrupts its ability to form Myddosomes. *J Biol Chem* 2011, 286:11875-11882.

33. **Kenny EF, O'Neill LA**: Signalling adaptors used by Toll-like receptors: an update. *Cytokine* 2008, 43:342-349.

34. **Manna P, Ghosh M, Ghosh J, Das J, Sil PC**: Contribution of nano-copper particles to in vivo liver dysfunction and cellular damage: Role of I B␣/ NF- B, MAPKs and mitochondrial signal. *Nanotoxicology* 2011, 552124:1-21.

35. **O'Neill LA, Fitzgerald KA, Bowie AG**: The Toll-IL-1 receptor adaptor family grows to five members. *Trends Immunol* 2003, 24:286-290.

36. **Justyna Rzepecka, Sebastian Rausch, Christian Klotz, Corinna Schnöller, Tina Kornprobst, Jana Hagen, Ralf Ignatius, Richard Lucius, Susanne Hartmann,** 2009 Calreticulin from the intestinal nematode Heligmosomoides polygyrus is a Th2-skewing protein and interacts with murine scavenger receptor-A *Mol. Immunol*. 46 ;1109–1119

37. **Venugopal, P. G., Nutman, T. B., and Semnani, R. T**. (2009). Activation and regulation of toll-like recep- tors (TLRs) by helminth parasites. *Immunol. Res*. 43, 252–263.

38. **Everts, B., Smits, H. H., Hokke, C. H., and Yazdanbakhsh, M**. (2010). Helminths and dendritic cells: sens- ing and regulating via pattern recog- nition receptors, Th2 and Treg responses. *Eur. J. Immunol*. 40, 1525–1537.

39. **Kane, C. M., Cervi, L., Sun, J., McKee, A. S., Masek, K. S., Shapira, S., Hunter, C. A., and Pearce, E. J.** (2004). Helminth antigens modulate TLR- initiated dendritic cell activation. *J. Immunol*. 173, 7454–7461.

40. **van Liempt, E., van Vliet, S. J., Engering, A., Garcia Vallejo, J. J., Bank, C. M., Sanchez-Hernandez, M., van Kooyk, Y., and van Die, I.** (2007). Schisto- soma mansoni soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation. *Mol. Immunol*. 44, 2605–2615.

41. **Ritter, M., Gross, O., Kays, S., Ruland, J., Nimmerjahn, F., Saijo, S., Tschopp, J., Layland, L. E., and Prazeres da Costa, C.** (2010). Schistosoma man- soni triggers Dectin-2, which acti- vates the Nlrp3 inflammasome and alters adaptive immune responses. *Proc. Natl. Acad. Sci.* U.S.A. 107, 20459–20464.

42. **Whelan, M., Harnett, M. M., Hous- ton, K. M., Patel, V., Harnett, W., and Rigley, K. P.** (2000). A filarial nematode-secreted product signals dendritic cells to acquire a pheno- type that drives development of Th2 cells. *J. Immunol*. 164, 6453–6460.

43. **Goodridge, H. S., Marshall, F. A., Else, K. J., Houston, K. M., Egan, C., Al-Riyami, L., Liew, F. Y., Harnett, W., and Harnett, M. M.** (2005). Immunomodulation via novel use of TLR4 by the filarial nematode containing ES-62.J. 284–293. phosphorylcholine-secretedproduct, *Immunol.* 174,

44. **Goodridge, H. S., McGuiness, S., Hous- ton, K. M., Egan, C. A., Al-**

Riyami, L., Alcocer, M. J., Harnett, M. M., and Harnett, W. (2007). Phospho- rylcholine mimics the effects of ES-62 on macrophages and dendritic cells. *Parasite Immunol*. 29, 127–137.

45. **Moreels TG, Nieuwendijk RJ, De Man JG, De Winter BY, Herman AG, Van Marck EA:** Concurrent infection with Schistosoma mansoni attenuates inflammation induced changes in colonic morphology, cytokine levels, and smooth muscle contractility of trinitrobenzene sulphonic acid induced colitis in rats. *Gut* 2004, 53:99-107.

46. **Brattig, N. W., C. Bazzocchi, C. J. Kirschning, N. Reiling, D. W. Buttner F. Ceciliani, F. Geisinger, H. Hochrein, M. Ernst, H. Wagner, et al**. 2004. The major surface protein of Wolbachia endosymbionts in filarial nematodes elicits immune re- sponses through TLR2 and TLR4. *J. Immunol.* 173: 437– 445

47. **Taylor,M.J.,H.F.Cross,andK.Bilo**.2000.Inflammatoryresponsesinducedb ythe filarial nematode Brugia malayi are mediated by lipopolysaccharide-like activity from endosymbiotic Wolbachia bacteria. *J. Exp. Med*. 191: 1429 –1436.

48. **Medzhitov, R., Preston-Hurlburt, P., and Janeway, C.A., Jr** (1997) A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* **388:** 394– 397

49. **Alvarez, J. I**. 2005. Inhibition of Toll-like receptor immune responses by micro- bial pathogens. *Front. Biosci.* 10: 582–587.

50. **Akira S, Takeda K.** Toll-like receptor signalling. Nat Rev Immunol. 2004; 4:499–511. [PubMed: 15229469]

51. **Agrawal, S., Agrawal, A., Doughty, B., Gerwitz, A., Blenis, J., Van Dyke, T., Pulendran, B.,** 2003. Cutting edge: different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated pro- tein kinase and c-Fos. *J. Immunol*. 171, 4984–4989.

52. Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science. 2003; 301:640–3. [PubMed: 12855817]

53. **Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E, et al.** LPS-TLR4 signaling to IRF-3/7 and NF-kB involves the toll adapters TRAM and TRIF. *J Exp Med*. 2003; 198:1043– 55. [PubMed: 14517278]

54. **O'Neill LA, Fitzgerald KA, Bowie AG**. The Toll-IL-1 receptor adaptor family grows to five members. *Trends Immunol*. 2003; 24:286–90. [PubMed: 12810098]

55. **Delhalle S, Blasius R, Dicato M, Diederich M**. A beginner's guide to NF-kB signaling pathways. *Ann N Y Acad Sci*. 2004; 1030:1–13. [PubMed: 15659775]

56. **Semnani RT, Sabzevari H, Iyer R, Nutman TB**. Filarial antigens impair the function of human dendritic cells during differentiation. *Infect Immun*. 2001; 69:5813–22. [PubMed: 11500459]

57. **Agrawal, S., Agrawal, A., Dougthy, B., Gerwitz, A., Belnis, J., Van Dyke, T., and Pulendran, B**. (2003). Different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modula- tion of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *J. Immunol*. 171, 4984–4989.

58. **Correale, J., and Farez, M**. (2009). Helminth antigens modulate immune responses in cells from Multiple Sclerosis patients through TLR2-dependent mechanisms. *J. Immunol*. 183, 5999–6012.

59. **Layland, L. E., Rad, R., Wagner, H., and da Costa, C. U.** (2007). Immunopathology of schistosomia- sis is controlled by antigen-specific regulatory T cells primed in the presence of TLR2. *Eur. J. Immunol*. 37, 2174–2184.

60. **Kamda, J. D., and Singer, S. M.** (2009). Phosphoinositide 3-kinase-dependent inhibition of dendritic cells interleukin-12 production by Giardia lamblia. *Infect. Immun*. 77, 685–693.

61. **Goodridge, H. S., Marshall, F. A., Else, K. J., Houston, K. M., Egan, C., Al-Ryami, L., Liew, F. Y., Harnett, W., and Harnett, M. M.** (2005). Immunomodulation via novel use of TLR4 by the filiarial nematode phosphorylcholine- containing secreted product, ES-62. *J. Immunol*. 174, 284–293

62. **Subash Babu, R. Anuradha, N. Pavan Kumar, P. Jovvian George, V.**

Kumaraswami, and Thomas B. Nutman Toll-Like Receptor- and Filarial Antigen-Mediated, Mitogen-Activated Protein Kinase- and NF-□B-Dependent Regulation of Angiogenic Growth Factors in Filarial Lymphatic Pathology *Infect. Immun.* 2012, 80(7):2509. DOI: 10.1128/IAI.06179-11

63. **Semnani RT, Nutman TB**. 2004. Toward an understanding of the inter-action between filarial parasites and host antigen-presenting cells. *Immunol. Rev*. 201:127–138

64. **Zhang G, Ghosh S**. Negative regulation of toll-like receptor-mediated signaling by Tollip. *J Biol Chem*. 2002; 277:7059–65. [PubMed: 11751856]

65. **Butcher BA, Kim L, Johnson PF, Denkers EY**. Toxoplasma gondii tachyzoites inhibit proinflammatory cytokine induction in infected macrophages by preventing nuclear translocation of the transcription factor NF-kB. *J Immunol*. 2001; 167:2193–201. [PubMed: 11490005]

66. **Shapira S, Harb OS, Caamano J, Hunter CA**. The NF-kB signaling pathway: immune evasion and immunoregulation during toxoplasmosis. *Int J Parasitol*. 2004; 34:393. [PubMed: 15055212]

67. **Taylor MJ, Cross HF, Bilo K**. Inflammatory responses induced by the filarial nematode Brugia malayi are mediated by lipopolysaccharide-like activity from endosymbiotic Wolbachia bacteria. *J Exp Med*. 2000; 191:1429–36. [PubMed: 10770808]

68. **Brattig NW, Bazzocchi C, Kirschning CJ, Reiling N, Büttner DW, Ceciliani F, et al**. The major surface protein of Wolbachia endosymbionts in filarial nematodes elicits immune responses through TLR2 and TLR4. *J Immunol*. 2004; 173:437–45. [PubMed: 15210803

69. **Hise AG, Daehnel K, Gillette-Ferguson I, Cho E, McGarry HF, Taylor MJ, et al.** Innate immune responses to endosymbiotic Wolbachia bacteria in Brugia malayi and Onchocerca volvulus are dependent on TLR2, TLR6, MyD88, and Mal, but not TLR4, TRIF, or TRAM. *J Immunol*. 2007; 178:1068–76. [PubMed: 17202370]

70. **Everts, B., Perona-Wright, G., Smits, H. H., Hokke, C. H., van der Ham, A. J., Fitzsimmons, C. M., Doenhoff, M. J., van der Bosch, J., Mohrs, K., Haas, H., Mohrs, M., Yazdanbakhsh, M., and Schramm,**

G. (2009). Omega-1, a glycoprotein secreted by Schistosoma mansoni eggs drives Th2 responses. *J. Exp. Med*. 206, 1673–1680.

71. **Candolfi E, Hunter CA, Remington JS**. Mitogen- and antigen-specific proliferation of T cells in murine toxoplasmosis is inhibited by reactive nitrogen intermediates. *Infect Immun*. 1994; 62:1995–2001. [PubMed: 8168967]

72. **Dai WJ, Gottstein B**. Nitric oxide-mediated immunosuppression following murine Echinococcus multilocularis infection. *Immunology*. 1999; 97:107–16. [PubMed: 10447721]

73. **Schleifer KW, Mansfield JM**. Suppressor macrophages in African trypanosomiasis inhibit T cell proliferative responses by nitric oxide and prostaglandins. *J Immunol*. 1993; 151:5492–503. [PubMed: 8228241]

74. **Goodridge HS, Marshall FA, Else KJ, Houston KM, Egan C, Al-Riyami L, et al.** Immunomodulation via novel use of TLR4 by the filarial nematode phosphorylcholine-containing secreted product, ES-62. *J Immunol*. 2005; 174:284–93. [PubMed: 15611251]

75. **Jenkins SJ, Hewitson JP, Ferret-Bernard S, Mountford AP**. Schistosome larvae stimulate macrophage cytokine production through TLR4-dependent and -independent pathways. *Int Immunol*. 2005; 17:1409–18. [PubMed: 16186163]

76. **Harnett, W., and Harnett, M. M.** (2009). "Immunomodulatory activity and therapeutic potential of the filarial nematode secreted product, ES-62," in Pathogen-derive Immunomodualtory Molecules, ed P. G. Fallon (New York, NY; Landes Bioscience and Springer Science), 88–92.

77. **Velupillai, P., Secor, W. E., and Horauf Harm, A. M.** (1997). B-1 cell (CD5+B220+) outgrowth in murine schistosomiasis is genet- ically restricted and is largely due to activation by polylac- tosamine sugars. *J. Immunol.* 158, 338–344.

78. **van der Kleij, D., Latz, E., Brouwers, J.F., Kruize, Y.C., Schmitz, M., Kurt-Jones, E.A., Espevik, T., de Jong, E.C., Kapsenberg, M.L., Golenbock, D.T., Tielens, A.G.M., Yazdanbakhsh, M.,** 2002. A novel host-parasite lipid cross- talk. Schistosomal lyso-phosphatidylserine acivates Toll- like receptor 2 and affects immune polarization. *J. Biol. Chem.* 277, 48122–48129