Filarial infections, which are widespread in several regions of the world, constitute a very heterogenous group of parasitic diseases. Some of them like Wuchereriaiasis, Onchocerciasis may cause severe complications. The immunological study of filariasis was published in 1916 by Rodhain and Van Den Branden. Since then, there has been a large number of publications on the subject.

The parasites and diseases

Filarial parasites are complex organisms both structurally and in their life cycle characteristics. A precise knowledge of the life cycle and biology of the parasite is important because the immune response to each stage in the worm's life cycle varies, and the clinical manifestations which are probably the consequence of the immune response to these parasites are related to particular stages in the life cycle.

Filariasis is caused by tissue dwelling nematodes of the super family Filarioidea. It is a chronic infection leading to extensive morbidity with little mortality (Piessens et al 1982, Ottensen 1980, Nelson 1979). Five species that infect man appear in the circulation as larval forms known as microfilariae (mf). The mf of the sixth species, *Onchocerca volvulus*, do not usually enter the circulation but migrate freely in the subcutaneous tissues and dermis. Adult Wuchereria and Brugia species inhabit different lymphatic vessels, while, adult *Onchocerca* and *Loa* spp. develop in the subcutaneous tissues of their hosts. The reasons for such tropism of filarial parasites for certain tissues or organs in their final hosts are poorly understood. In addition to the
Wuchereria bancrofti MF showing sheath

10 X

45 X
six species mentioned above, a number of filarial parasites that infect other animals are occasionally reported in humans. Filarial parasites that commonly infect man are shown in Table (I).

**Life cycle**

Adult filarial worms live in the tissue or body cavities of a vertebrate host. The females produce partially embryonated eggs that before or at the time of oviposition contain embryos that uncoil and become delicate snake like organisms called microfilariae (mf). If the egg membrane elongates to accommodate itself to the elongated embryo, the latter is said to be sheathed; if the shell ruptures and sets free a naked embryo, the latter is said to be unsheathed. The mf are modified for a prolonged existence in an undeveloped state in the blood or skin. The transmission is affected by the uptake of the first stage larvae (mf) into hematophagous arthropods as they feed on the infected host. In appropriate arthropods the mf migrate out of the digestive tract into the hemocoel, and in suitable locations, frequently the thoracic muscles develop without multiplication into second and later into more advanced third stage larvae. The mature larvae migrate down into the hemocoelic cavity in the labium and escape into the vertebrate host's skin when the arthropod takes its next blood meal. The infective larvae mature into adult worms in a susceptible host. This process takes place over a period of several months during which the parasite molts twice. There are no free living stages and both the mf and adults may persist for a long time. Life cycle of the parasite is represented in (Fig. 1).
Fig 1: Life cycle of the parasite is represented below.
The following are some particular characteristics in the life cycle.

Very little is known about the whereabouts and migration route of the infective larvae and fourth stage larvae in the human host. It is likely that several life cycle stages are present in individuals who live in endemic areas where transmission occurs continually. Normally elongated mf are discharged by gravid female worms. However, occasionally, uncoiled ovoidal embryos escape from the female worms. Nothing is known about the fate of these embryos. It is not known why microfilariae of some filarial species possess a sheath. The biochemical and physiological properties of the microfilarial sheath are also unknown. The sheath of mature *Brugia phanagi* mf binds Concanavalin A and wheat germ agglutinin indicating the presence of N-acetylglucosamine and galactose or mannose (Furman and Ash 1983). The same arthropod vector may harbour simultaneously infective larvae of several filarial species (Voelker and Garms 1972).

The level of mf in the peripheral blood does not directly correlate with the number of adult females in the host (Worms 1972, Pachehco 1974) and does not represent the total number of microfilariae produced by female worms (Beaver et al 1974, Denham et al 1972). Some of the microfilariae may be diverted to other organs, like lungs, spleen, kidney and liver (Grove 1979, Van Marck 1983). There appears to be a threshold in the level of circulating mf at a given time of infection that cannot be altered by the addition or removal of mf. Indeed, the addition of microfilariae by transfusion or removal by repeated bleeding failed to change the pre-existing level of microfilaraemia (Wong 1964, Greenough and Buckner 1969).
Table I

Filarial parasites that commonly infect man

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Location of adults</th>
<th>Nature of Microfilariae</th>
<th>Vectors</th>
<th>Geographic distribution</th>
<th>Common disease symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brugia malayi</em></td>
<td>Lymphatics, frequently above diaphragm</td>
<td>Sheathed, showing periodic or subperiodicity depending on strain.</td>
<td><em>Mosquitoes</em>: <em>Mansonia</em>, <em>Anopheles</em>, <em>Aedes</em> spp.</td>
<td>For East, India to Japan.</td>
<td>Adenolymphangitis, elephantiasis.</td>
</tr>
<tr>
<td><em>Onchocerca volvulus</em></td>
<td>Subcutaneous nodules</td>
<td>Unsheathed, throughout cutaneous lymphatics, some time in blood stream</td>
<td>Black flies: <em>Simulium</em> spp.</td>
<td>Africa, Central and South America.</td>
<td>Dermatitis, skin nodules, blindness.</td>
</tr>
<tr>
<td><em>Loa loa</em></td>
<td>Migrating in cutaneous and subcutaneous tissues</td>
<td>Sheathed, showing diurnal periodicity</td>
<td>Tabanid flies: <em>Chrysops</em> spp.</td>
<td>Western and Central Africa</td>
<td>Calabar swellings</td>
</tr>
<tr>
<td><em>Dipetalonema perstans</em></td>
<td>In body cavities</td>
<td>Unsheathed, nonperiodic</td>
<td><em>Culicoides</em> spp.</td>
<td>Africa, Central and South America.</td>
<td>Usually benign</td>
</tr>
<tr>
<td><em>Mansonella nazzardi</em></td>
<td>In body cavities</td>
<td>Unsheathed, nonperiodic</td>
<td><em>Culicoides</em> spp.</td>
<td>Central and South America.</td>
<td>Usually benign.</td>
</tr>
</tbody>
</table>
In some species, the number of circulating mf in peripheral blood fluctuates in a predictable rhythmic pattern over a 24 hr period. This phenomenon is known as circadian periodicity (Manson 1888, Hawking 1967, Anderson et al 1975). The factors that control periodicity are probably physiological and are poorly defined. But mechanisms controlling the release of mf from numerous species of filarial worm have been studied experimentally for many years by Hawking and Thurston (1951a,b), McFadzean and Hawking (1956), Rowlands (1956) and Hawking (1965), who found that during daylight hours Mf of W.bancrofti collected in the pulmonary vessels proximal to the pulmonary arterioles. The same localization of mf occurred when a patient breathed air with a high oxygen content. Experimental work has shown that other and different species of filariae release their mf in response to varying stimuli which include alternation of the arterial oxygen tension and changes in the body temperature. The nocturnal release rhythm shown by most strains of W.bancrofti mf has been altered and inverted to diurnal release if the patients sleep during the day time.

Clinical spectrum of the lymphatic filariasis

Bancroftian and Brugian filariasis are characterized by a wide spectrum of clinical manifestations, the signs and symptoms often differing from one endemic area to another. The spectrum of clinical manifestations stretches from tropical eosinophilia syndrome, lymphatic obstruction and filarial fevers, to asymptomatic microfilaraemia. There are many individuals
with no clinical manifestations or indication of filarial infection, though they are exposed to infective bites.

**Endemic normals**

Individuals living in endemic regions but with absolutely no clinical or parasitological evidence of infection form a particularly important group for study. This group may be called as "exposed but not infected" individuals (Ottensen et al 1977). Recent studies however suggest that a subset of these individuals may in fact remain clinically asymptomatic and parasitologically undetectable with available diagnostic techniques.

**Asymptomatic microfilaraemia cases**

There is also another group of people entirely asymptomatic but have the presence of microfilariae circulating in the peripheral blood. People with this manifestation of filariasis have been called "asymptomatic microfilaraemics". It is unclear as to how long they remain asymptomatic and at which time they move either to the right, with clinical manifestations or to left with clearance in this clinical spectrum, but their number in any endemic region is quite considerable.

**Filarial fevers**

The most common of the symptoms in the filarial syndrome, is the characteristic recurrent episodes of filarial fever in many infected individuals. These febrile episodes are accompanied by inflammation of lymphatic tissues
(ie. lymphadenitis and lymphangitis), that occur roughly 2-6 times per year, lasts for about a week and then subside, spontaneously for reasons which are just as unclear as are the factors which initiate the episodes. Individuals with these filarial fevers may or may not be microfilaraemic. The importance of understanding the pathogenesis of this condition is very essential because this stage leads to lymphatic pathology.

**Lymphatic obstruction**

The chronic stage of filariasis develops in 10 to 15 years and only a small proportion of the infected community is affected. The chronic clinical features of Brugian filariasis are quite distinct from those of bancroftian filariasis. In Brugian filariasis the leg below the knee or less frequently the arm below the elbow characteristically affected. This is a surprising feature since, during the acute stage, the affected lymph nodes are usually located in the inguinal region. In most cases only the foot and the distal part of the leg is affected. The size of the affected leg is usually more than twice its original size. Typically the swelling does not progress beyond the knee, preserving more or less the contour of the affected knee. Genitalia involvement and chyluria have not been reported except in areas where Brugian and Bancroftian filariasis occur together.

In bancroftian filariasis, hydrocoele and sometimes chylocele are the common signs in endemic areas followed by lymphoedema and elephantiasis of the whole leg, arm, scrotum, vulva, breast, in the order of decreasing frequency. In the initial stage, the swelling can be best observed around the
ankle. It gradually spreads to the back of the foot, the calf and thigh. The size of the affected extremity may easily be increased to more than thrice its original size. Chyluria is observed in some endemic areas. Not infrequently it occurs episodically and is more pronounced after a heavy meal. The chronic stage of filariasis is often considered as a static end stage when the adult worms are dead and therefore, mf are absent or rarely observed in the blood.

Tropical pulmonary eosinophilia (TPE)

This is perhaps the best example of occult filariasis. It was first formally described by Frimodt and Barton in (1940) although in 1939 Meyer and Koawenoer had demonstrated mf in the inguinal lymph nodes of individuals with eosinophilia and asthma. Five years later, Van der Sar and Hartz (1945) demonstrated mf in the lymph nodes and spleens of their patients. Ottensen et al (1979) and Jayewardene and Wijayaratam (1968) showed that, TPE was a disease caused by human filarial parasites and not by animal filarial parasites as was then believed. TPE also has extrapulmonary manifestations. There are ethnic differences in the prevalence. It is more commonly seen on the Indian subcontinent although Bancroftian filariasis is endemic in many parts of the world. It can occur at any age and has been reported even in infants (Indrabai et al 1976). The disease can have acute or an insidious onset. Its clinical manifestations are usually cough, fever, chest pain, breathlessness, malaise and occasional abdominal pain. Examination of the chest reveals rhonchi and crepitations. Sometimes lymphadenitis, splenomegaly and hepatomegaly may also be observed. The natural course is marked by recurrences and relapses. Long
standing untreated cases progress to pulmonary fibrosis and respiratory insufficiency (Udwadia 1967). These patients have blood eosinophilia, raised erythrocyte sedimentation rates and there may be evidence of diffuse miliary lesions of increased bronchovascular marking in the chest X-rays especially in children (Ball 1950). Microfilariae are not demonstrated in the blood smears but can be found in the lungs, in the liver and lymph nodes on thorough examination (Joshi et al 1969). There is impairment of lung function with occurrence of both obstructive and restrictive defects (Kamat et al 1970). It responds to treatment with diethyl carbamazine (DEC) which is yet another indication of its filarial origin.

**Immunologic profile of individuals with filariasis**

Large volume of immunologic informations on human filariasis is available through the work of many investigators, who used techniques such as skin testing, various antibody assays, and in vitro methods of assessing lymphocytes responsiveness. It is clear that these infections elicit both humoral and cell mediated immune responses, in their human hosts. But there are atleast three distinctive features of this response which should be recognized before one considers the specific immunopathology associated with these diseases.

Foremost among these distinctive features is the fact that chronic filariasis (with the exception of the tropical eosinophilia syndrome) is characterized by a state of marked immunologic hypo-responsiveness to parasite antigens, and very prominent immuno-regulatory mechanisms
functioning apparently to "contain" or limit normal responsiveness to parasite antigens in infected individuals.

This "immune suppressed" state has been most clearly demonstrated in in vitro studies of monocyte function (Ottensen et al 1977, Piessens et al 1980). It has been shown that patients with chronic infection respond poorly or not at all to filarial antigens, but that their responses to other antigens and to mitogens remain normal. Further more, the lymphocyte responses in clinically infected children are normal where as the same is modulated or dammed down" by various suppressor mechanisms in adults (Ottensen et al 1977).

With respect to the specific mechanisms involved in this immunosuppression, data are much less complete (Piessens et al 1980). The biologic importance of this parasite specific diminished host responsiveness has not been defined with certainty but it may well play a role in mechanisms involved in the long term tolerance of the parasite by host, which is of course, the most fundamental aspect of parasitism itself.

A second feature of the immunologic profile of these patients derives from the prolonged persistence of the living parasites within the host. Antigens are shed or secreted almost continuously by the parasites and in the presence of antibody, immune-complexes are formed. Several groups have documented the fact that a large percentage of patients with filariasis have either circulating antigens or circulating immune-complexes (Lambert 1978). These complexes play an important role in initiating or modulating numerous aspect of the overall host response to these parasites.
The third distinctive feature of the host response in filariasis is a feature shared with other chronic helminth infection namely, the prominent involvement of immediate hypersensitivity (IH) immune mechanisms. Serum IgE levels are elevated, eosinophils are prominent, basophils and mast cells can be shown to be sensitized with specific reaginic antibody (Ottensen et al 1979). Similar responses are not regularly elicited by either protozoal parasites or other infectious agents, but how these immediate hypersensitivity mechanisms function during chronic filarial infection is still a mystery. Indeed, the fact that most infected individuals possess high levels of IgE and sensitized basophils, and have parasite antigens but still are not allergically symptomatic is quite remarkable in itself.

Pathology

The pathology of filariasis breaks down generally into two categories, one associated with adult worms, and the other associated with microfilariae. However, for the tropical eosinophilia syndrome the immunologic determinants responsible for the pathogenesis of each of the clinical states outlined in Fig.2 have been difficult to define (Fig. 2).

Microfilaria related pathology

While it is possible that mf do play a role in inducing lymphatic inflammation, most of the recognized pathology-associated with this stage of the parasite results from tissue reactions around these parasites which have been cleared from the blood. In microfilaremic individuals there is a continual production of mf. Clearance of these mf presumably takes place consistently
Fig. 2 Clinical spectrum of the lymphatic filariasis.
in the lungs, liver and spleen but the clearance appears to be unassociated with any definable clinical symptoms. Occasionally aberrent mf will be found in the breast, subcutaneous tissue or other sites where they elicit a granulomatous response which can evoke a systematic presentation (Earle 1942, Saxena et al 1975, Winckel et al 1953). It is likely that there are immunological regulatory mechanisms responsible for the lack of overt responsiveness to the microfilarial stage of the parasite in most patients with filariosis, but these are yet to be defined.

The situation however, is entirely different in patients with the tropical eosinophilia syndrome. These patients will constitute less than 1% of the all individuals with filariosis. They appear to be immunologically hyper-responsive to all filarial antigens especially to those derived from microfilariae. Antifilarial antibodies of all classes are markedly elevated and IgE and eosinophils levels can be as high in this condition as in any pathologic state except for certain neoplasms (Neva and Ottensen 1978). Both the clinical state as well as the laboratory findings suggest a prominent role for immediate hypersensitivity responses in the pathogenesis of the syndrome. Recent studies on the histamine release responses from patient's basophils as in vitro correlate of reagin mediated allergic reactions, have allowed to compare the specificities of the IgE antibodies of these individuals with those of patients with other forms of filariosis. It has become clear that while all filariosis patients have large amount of IgE antibody directed against adult worm antigens, patients with tropical eosinophilia show a special hypersensitization to antigens derived from the microfilarial stage of the parasite (Ottensen et al 1979). These observations have given strong
support to the original speculations of others (Danaraj et al 1966, Joe 1962). Wong and Guest (1969) reported that tropical eosinophilia is a form of "occult filariasis" in which the absence of circulating microfilariae reflects an immunologic hyper-responsiveness on the part of the host which results in effective clearance of this stage of parasite from the blood. Much of this clearance is probably mediated by IgE antibodies and effected preferentially by the lungs. The asthmatic symptoms are the likely results of pulmonary allergic responses mediated by specific IgE antibodies directed against the microfilariae. It is not clear why some people will become allergic and others asymptomatic while clearing the microfilariae.

Lymphatic pathology

Most of the pathology associated with Bancroftian, Brugian filariasis are limited to the lymphatics. While the details of the pathogenesis are obscure and probably complex, the various expressions of this pathology are really quite simple. Incompetence of the draining lymphatics leads first to lymphoedema and then to either elephantiasis of the limbs, breasts, or genitalia or leakage of chyle into the urine (chyluria). The location of the lymphatic abnormalities determines the site and the type of pathology expressed.

Normal lymph vessels are delicate, endothelium lined channels which lead from an extensive network of peripheral lymph capillaries through a series of collecting vessels and intermediate lymph nodes to the large cysterna chyli and thoracic duct which empties into the venaacava. Lymph
flow is determined primarily by contraction of skeletal muscles and depends on a system of valves as extensive as that found in the veins of the vascular tree. In filariasis adult worms reside within these lymphatic vessels generally in the afferent approaches or cortical sinuses of the lymph nodes. The parasites first cause a dilatation of the lymphatic followed by hypertrophy of the vessel wall. There is an endothelial and connective tissue proliferation with polypoidal growths protruding into the lumen, but so long as the worm remains alive, the vessels appear to remain patent (O’Connor 1932). This patency, however, does not assure normal lymphatic function as lymphographic studies document clearly the development of the characteristic tortuosity of the lymph vessels with loss of valvular functions and backflow of lymph during this pre-obliterrative phase (Cohen et al 1961). Thus lymphstasis may occur during the life of the worm and results in early lymphedematous changes in the affected limb of genital organs.

It is uncertain, what causes adult worms to die, but their death is associated with certain distinctive pathologic changes (O’Connor 1932). An area of necrosis develops about the parasite, resulting both from degeneration of inflammatory cells and dissolution of parasite material. A granulomatous reaction ensures with the formation of foreign body giant cells and a concomitant infiltration of eosinophils. Collagen deposition is seen and the parasite, after fragmentation, either becomes completely absorbed or partially calcified. It is during these pathologic reactions within the vessel that lymphatic obstruction occurs. Subsequently, collateral vessels develop to aid the passage of lymph but recanalization of the obstructed tracts can also been seen, especially the early phases of lymphatic obstruction.
These observations which were made primarily on human autopsy material have been substantiated to a large degree in experimental model systems such as the cat (Denham and Mc Greovy 1977, Ewert et al 1972) or dog (Schacher et al 1973, Schacher and Sahyoun 1967) infected with Brugia species. However, there has been one important issue raised in these animal studies which could not be noted in the autopsy series; namely, the potential importance of molting fluids or products of the preadult (L₂ and L₄) and larval stages in initiating the lymphatic inflammatory damage observed. Both radiographic and direct anatomic evaluations of the pathology in these animals have indicated that the local lymphatic inflammation and distortion occur primarily when the infecting parasites are molting and releasing the highly antigenic material which is shed at this time (Schacher and Sahyoun 1967). Obviously, death of adult parasites could intensify the local inflammation but death of the parasites alone was incapable of inducing the same level of inflammatory response that the molting products did. These observations assume a relatively greater importance when we consider the pathogenesis of the acute filarial syndrome.

Funiculitis, epididymitis and orchitis are also frequently observed in filarial infection in male. The inflammation at each of these sites occurs as a direct result of parasites in the associated lymphatics. Moreover in addition to the various types of inflammatory responses found in these lymphatics, there is often an associated phlebitis which has been described in detail by Lpchenberg (1957). There is little question about the mechanisms underlying the inflammation of the genital lymphatics and the progression of obstructive pathology which are similar or identical to those responsible for lymphatic inflammation found else where.
Unfortunately, neither the immunologic mechanisms nor the precise etiologic agents (molting fluids or dead adult parasites) involved in the pathogenesis of these lymphatic and Brugian filariasis are understood as yet. Indeed, clues have been difficult to come by. Two recent sets of observations however, suggest that there may be a potential role for cell mediated immune response in the development of this pathology. First, studies of patients with Brugian filariasis, Piessens et al (1980) found that lymphocyte responsiveness to certain filarial antigens was significantly greater in those patients with lymphatic pathology than others without it. Earlier studies on Bancroftian filariasis patients using similar techniques had not shown such differences (Ottensen et al 1977) but the implications of Piessen's (1980) findings are both clear and potentially important. Further study is needed to resolve this important issue.

Second, even more direct evidence for the role of cellular immune mechanisms in the pathogenesis of lymphatic pathology has come from work in Jirads infected with Brugia phanagi (Keli et al 1986). In this model chronic administration of antithymocyte serum to infected animals diminished the lymphatic pathology, which normally develops after infections of these animals. Although the interpretation of this type of experiment is not completely straightforward, the potential role for cell mediated immunity in the development of lymphatic pathology is clearly indicated by these two sets of observations.
Immune response in human filariasis

Bancroftian and Brugian mf appear in blood only at night with peak concentration between 24 and 02 hours. The usual practice of screening 20 cm³ of night blood for mf is extremely poor and often unreliable. To improve the parasite detection potential, many immunological tests have been developed and evaluated in the field. These tests though had their limitations, (Kagan 1963, Desowitz et al 1976, Weiss and Degremont 1976, Grove et al 1978) have provided a substantial body of information on the immune response in filariasis.

A. Skin tests

Filarial skin test was the foremost of the tests employed for immunodiagnosis. At least 13 different filarial parasites have been used for antigen preparation (Kagan 1963). Most popular among them, was D.immitis saline extract which showed specific immediate hypersensitivity response in 21 out of 23 filariasis patients, none of the controls reacted. Taliaferro and Hoftman (1930) and Fairley (1931, 1932) using aqueous extracts of D.immitis have demonstrated immediate skin reaction in elephantiasis patients.

Sawada et al (1968, 1969) have claimed that the highly purified D.immitis (DI) antigen is useful in the detection of lymphatic filariasis. This antigen contains approximately twelve proteins detectable by electrophoresis. The D.immitis antigen was evaluated in many countries by WHO and the results were equivocal (Chandra et al 1974, Smith et al 1971). The sensitivity of this antigen was questioned by Grove et al (1977) who stated that
B.malayi skin test antigen was much more sensitive. They further claimed that D.immitis antigen was more reactive in patients with malayan filariasis rather than in bancroftian filariasis. Because of these limitations, further work with D.immitis antigen was discontinued. The overall consensus is that for W.bancrofti infection, D.immitis skin test antigen is not as sensitive as that made from B.malayi.

There are reasons to believe that sufficient antigenic material from W.bancrofti will not be available in years to come, and the choice naturally falls on the use of other human lymphatic filarial worm ie., B.malayi. Although cats, dogs and monkeys (Murthy et al 1983a, Schacher, et al 1969, EL Bihari and Ewert 1971), have been found to be suitable hosts for B.malayi infection, their large size has discouraged their use. This infection has now been successfully transmitted to small laboratory rodents, Mastomys natalensis and Gerbil (Sanger et al 1981b, Murthy et al 1983b, Ash and Riley 1970, Murthy et al 1987). These small rodents have obvious advantages over bigger animals. Since the small animals allow the harvest of sufficient parasite material, the antigen from B.malayi has extensively been used to develop a sensitive, specific quick and easy tests (Chandra et al 1986, Kaliraj et al 1979).

Grove et al (1977) have employed saline extracts of mf, adult worms and L₃ of sub periodic strain of B.malayi and evaluated these antigens in skin test in Phillipines. They found over 90% of the infected patients reacted to all three antigens in filaria skin test (FST), while D.immitis antigen was less sensitive.
Later, the high sensitivity and specific reactivity of *B. malayi* L₃ antigen was endorsed by Chandra *et al* (1978) and Katiyar *et al* (1985). They carried out trails in *W. bancrofti* endemic area and found that L₃ antigen was highly reactive in bancroftian subjects. The specificity of the antigen was examined against *Ascaris* and *Trichuris* in Kashmir, a filaria non-endemic area and against Guinea worm (*Dracunculus medenisis*) in Rajasthan, a filaria free zone (Katiyar *et al* 1985). The cross-reactivity was insignificant suggesting the specificity of antigen for filaria alone. It was further claimed that the powdered antigen could be stored for longer duration. However, the reconstituted antigen had short life (Murthy *et al* 1988). After the extensive trials covering over 5000 subjects from filaria endemic areas and non-endemic areas and using improved parasitological techniques they inferred that the antigen detects mf carriers, clinical, prepatent and latent cases and also the occult cases of filariasis. The test procedure is very simple and the results can be pronounced on the spot.

Investigators further stated that the skin test antigen has significant value in measuring the exposure rate. As far as individual diagnosis is concerned, they concluded that, in patients exhibiting vague filaria related symptoms, positive skin reaction would confirm filariasis and a negative reaction in suspected cases would exclude filariasis.

**Cell mediated immune reactions in human filariasis**

One of the *in vivo* parameters of specific cell-mediated immune response is the intradermal skin test. A positive test is observed as an induration at the site of antigen administration. It becomes prominent after 48-72 hours, and hence called delayed type hypersensitivity. The induration is as a result of mono-nuclear cell infiltration.
Delayed skin reaction to filarial antigen was demonstrated by various workers in filariasis patients (Fairley 1931, Bozicevich and Hutter 1944, King 1944 and Sanders et al. 1946). However, the role played by cell-mediated immunity in human filariasis has not received much attention.

Many new techniques have been developed for the study of cell mediated immunity. Ottensen et al. (1977) were the first ones to use these techniques and reported on the role played by cell-mediated immunity in filariasis. In a study conducted in Cook Islands, they performed lymphocyte transformation test on filariasis patients and endemic controls. They reported specific cellular unresponsiveness to filarial antigens in infected children. In adult population, controls as well as infected individuals, reacted relatively less vigorously. Using B. malayi antigens, it was possible to show that adults with microfilariae in circulation responded significantly less when compared with controls.

Piessens et al. (1980) conducted studies on sub-periodic B. malayi infection in Indonesia. Using microfilarial antigen they observed correlation between the presence of cellular immunity to microfilarial antigens and the absence of microfilaraemia. A state of antigen specific immune unresponsiveness during patent microfilaraemia was shown to be mediated by an adherent mononuclear type of suppressor cell. They also reported the presence of suppressor factors in sera.

Both the studies mentioned above were conducted on B. malayi infected patients. While chronic patients showed specific cellular unresponsiveness in
the study by Ottensen et al (1977), the microfilariaemic patients showed unresponsiveness to the filarial antigen in the study by Piessens et al (1980).

Raghunath, et al (1985) have studied the cell-mediated immune response in human filariasis. This study has been carried out by counting the percentage of T-lymphocytes in peripheral blood by E-rosette test and measurement of Leucocyte migration inhibition (LMI). Leucocytes were sensitized specifically with sonicated antigen of W.bancrofti and also with non-specific purified protein derivatives (PPD). Study revealed that T-cell population was maximum in patients with high mf count and chronic cases, in comparison to the patients with low mf count, endemic and non-endemic normals. Mean migration index (MI) with filarial antigen and PPD was found to be more in mf carriers (high counts) and chronic cases. Similarly MI was found to be more with PPD. Thus showing depressed immunological responses only in patients with high mf count and chronic cases. They could not explain the mechanisms by which specific and non-specific immunity were suppressed in bancroftian filariasis.

Mehta et al (1980) studied the suppression of mitogenic response to PHA and Con-A in bancroftian filariasis. They found that a marked reduction in blastogenic response of lymphocytes from cases of elephantiasis and microfilariae carriers to the mitogens PHA and Con-A, suggesting depression of cell-mediated immune responses in patients with filariasis. Peissens et al (1980) have studied antigen-specific suppressor cells and suppressor factors in human filariasis with B.malayi. They have investigated the mechanisms of specific immune unresponsiveness in humans with patent filarial infections, and found that the active suppression of immune responses is
directed against the parasite. There was no intrinsic inability of infected patients to react to parasite antigens.

Piessens et al (1982) have studied antigen-specific suppressor T-lymphocytes in human lymphatic filariasis. To determine whether hypereactivity to filarial antigens is associated with changes in subpopulations of T-lymphocytes that regulated the immune response, they have quantitated helper and suppressor T-cells in the blood of patients with B. malayi. Increased numbers of suppressor T-cells were present in 15 of 17 patients with microfilaraemia and in 6 of 11 patients with elephantiasis.

Gajanana et al (1981) have studied E and EAC rosette forming peripheral lymphocytes in human bancroftian filariasis in infected and non-infected groups living under similar environmental conditions. In infected group, neutropenia, eosinophilia and unaltered lymphocyte counts were observed. EAC rosette forming cells remained unchanged. T cells were less in the group showing clinical manifestations, where as microfilaraemia carriers did not show any alternation in T cell. However, non-T, non-B (null) cells showed an increase in number in untreated and mf carriers.

Mistry et al (1985) have studied the cellular immune competence in Bancroftian filariasis using both homologous (W. bancrofti) and heterologous (B. phanagni) antigens using Con-A, phytohaemagglutinin and unrelated stimulating agent such as PPD. This study reveals a marked variation in the lymphocyte reaction to W. bancrofti and B. phanagi microfilarial antigens in subjects at various stages of infection.
Also, immune unresponsiveness to the parasite antigens was marked only in nontreated microfilariae carriers. The pattern of reactivity to adult worm antigens was markedly different from that observed with microfilarial antigens.

Finally the suppression of response to mitogens and purified protein derivatives (PPD) was observed in filarial infection which was in accordance with the previous observations (Mehta et al. 1980, Alamelu et al. 1983).

Nutman et al. (1985) have studied the filarial parasite specific T-cell lines and induction of IgE synthesis. This study reveals the role of helminth parasite specific T-cells, and their soluble products in IgE production in human in vitro culture system. They have established that parasite specific T-cells as well as their products can provide IgE isotypes specific help. Furthermore, the soluble factors generated from these cells can induce the production of IgE in normal human cells not usually synthesizing measurable amount of IgE in vitro.

Mohapatra et al. (1988) have studied the host cellular response in bancroftian filariasis and observed that most subjects living in filariasis endemic areas, show evidence of hyper cellular reactivity, in contrast to marked hypo responsiveness in patients with filariasis. The immune hyperresponsiveness was towards mitogenic as well as antigenic stimuli.
Humoral antibody response

Many serological tests have been employed to detect antifilarial antibodies in filariasis. They include simple double diffusion test, complement fixation test, indirect haemagglutination test, fluorescent antibody and Enzyme linked Immunosorbent Assay for detecting antibody from the patient’s sera.

Complement fixation test

The complement fixation test for antifilarial antibodies was performed by Guerin 1924. Various studies conducted since then used this assay to study antifilarial antibodies. Kagan (1963) reviewed the complement fixation tests done in filariasis patients. On the basis of the results of various studies reviewed in his article, he concluded that the complement fixation test has a lot of limitations for use as a diagnostic test. The reaction was not specific and cross reaction could be detected with other helminths. Warren (1947) showed that filarial sera cross reacted with T.spiralis antigen in complement fixation test.

Precipitation tests

The precipitation test (PT) had some limited use in the diagnosis of filarial infections. Methodically the Ouchterlony test is generally used as screening test as a kind of preliminary test to immuno-electrophoresis. This test permits filariasis to be distinguished from other helminthic infections capable of causing eosinophilia. It takes several days to carry out immuno-
electrophoresis but the test results seem to be specific. Characteristic precipitin arcs are obtained with the homologous antigens and also with extracts of *D. viteae*, (Capron *et al* 1968) or *A. suum* (Gentilini *et al* 1972). This paradoxical finding is explained by the very analytical character of the method which can reveal antigenic fraction common to *Loa loa*, *W. bancrofti* or *O. volvulus* *D. viteae* and *A. suum*. These common fractions vary from case to case, so that one homologous antigen ultimately leads to specific results.

**Fluorescent antibody test (FAT)**

Chowdhury and Schiller (1962) for the first time applied fluorescent antibody test for the diagnosis of filariasis. Young (1973) used fragments of the microfilariae and infective larvae of *W. bancrofti* as the antigen for indirect fluorescent antibody test. None of the non-endemic control sera gave positive reaction where as all elephantiasis sera were positive. On the other hand about 40% of the endemic control sera gave a positive reaction. Weiss and Degremont (1976) found immunofluorescent test positive in 60% of Europeans returning after a stay in endemic areas. IFA and IHA tests when employed together could detect antibodies in 83% of patients. Hedge and Ridely (1977) demonstrated the antibodies in filarial sera using a sonicate of microfilariae as antigen. Parc *et al* (1978) devised a new method for the preparation of *W. bancrofti* antigen for fluorescence test. Wong and Suter (1979) measured antibodies against *D. immitis* adult Worms by IF. *Dipetalonema viteae* egg shell and uterine fluid were found to be very potent antigens for fluorescent antibody test (Diesfeld and Kirsten 1979). Gonzaga dos Santos *et al* (1976) have developed a new technique for the diagnosis of
Wuchereriasis. Microfilariae were treated with proteolytic enzyme and were used as antigen for immunofluorescent test. The technique for the preparation of antigen from microfilariae treated with papain is much simpler than that described by Coudert et al (1968) or by Thomas (1969) from sliced adult *D.immitis* and *D.viteae*. Kaliraj et al (1979) using sonicated *W.bancrofti* microfilariae, reported 100% and 54% positivity for antibodies in FAT test among clinical filariasis, and endemic normal sera. None of the 22 non-endemic normal sera showed positive reactions.

McGreevy et al (1980) studied antibodies against the microfilarial sheath of subperiodic *Brugia malayi* in a community of people living in an area endemic for malayan filariasis. Using the indirect fluorescent antibody (IFA) technique they determined the prevalence and titer of anti-sheath IgM and found that IgM was higher than anti-sheath IgG or IgA. Indra Khart et al (1983) studied the utility of *W.bancrofti* mf excretory and secretory antigen (ES) for the detection and identification of filarial antibodies by IFAT. They have used purified soluble ES antigen bound to CNBr-sepharose 4B beads. They could detect antibodies in 10/20 endemic normal, 19/20 microfilaraemia and all 20 clinical filarial sera. None of non-endemic sera showed the presence of antibody.

Sethumadavan et al (1988) have detected filarial antibodies using *B.malayi* and *Breinlia booliati* antigens in IFAT test. Antigen prepared from adult worms, microfilaria and infective larvae of sub-periodic *B.malayi* and *Breinlia booliati* were used in the IFA test to measure antibody levels in the sera of patients with *W.bancrofti* infection. Adult worm antigens detected
higher antibody levels than the other parasitic stages and in general \textit{B.malayi} antigens were more sensitive than \textit{Breinlia booliati} antigens. Senarath Dissanyake (1989) worked on the predictive value of IFAT in the diagnosis of \textit{W.bancrofti} infection. Rao \textit{et al} (1990) developed a direct fluorescence technique for the rapid detection of sheathed microfilariae in blood smears.

\textbf{Indirect haemagglutination test (IHA)}

IHA test was used for diagnosis of filariasis by Danaraj and Pacheco (1966) with an acid-soluble protein fraction prepared from \textit{D.immitis}. Takahashi and Sato (1976) used a fractionated and purified \textit{D.immitis} adult worm extracts. Fraction designed as FPSD$_4$ - showed high potency in IHA test. FPSD$_4$ on further analysis gave FPSDA antigen which was most reactive and specific in filariasis. Ottensen \textit{et al} (1977) used IHA test in endemic area. Antibodies were demonstrable in higher proportions in adult population compared with children. They could not establish association between the antibody titres and the clinical findings. Malkit Singh \textit{et al} (1980) reported the application of an indirect haemagglutination technique for the diagnosis of filarial infections in sera from persons exposed to \textit{B.malayi} and \textit{W.bancrofti} in endemic areas of Malaysia. They have used crude extract of a heterologous species, \textit{Breinlia booliati}. Kaliraj \textit{et al} (1982) have fractionated the soluble \textit{W.bancrofti} microfilarial antigens by Sephadex G-150 gel filtration followed by DEAE cellulose column chromatography in order to obtain active and specific antigenic fractions (mf S1, 2 & 3). The mf S$_3$ fraction was weakly reactive in indirect haemagglutination test, whereas the same was found to be highly reactive in Enzyme-linked immunosorbent assay (ELISA).
Counter immuno electrophoresis (CIE)

Apart from complement fixation test, IHA and IFA, antifilarial antibodies have also been detected using CIE. Of the various techniques employed for the visualization of precipitin antibody, counter immuno-electrophoresis (CIEP) seems to be the most sensitive test. Krupp (1974) found that CIEP was superior to immunodiffusion for the diagnosis of amoebiasis. Despomier et al (1974) reported CIEP was a sensitive, specific immunodiagnostic test for trichnosis. Desowitz and Una (1976) have detected antibodies in human and animal filariasis by CIEP with *D.immitis* antigens. They have detected precipitin antibodies in all 6 dogs and the 2 cats infected with *D.immitis* and also in the serum of 17 out of 24 individuals living in a hyperendemic subperiodic area.

Enzyme-linked immunosorbent assay (ELISA)

Kagan (1981) classified some of the immuno-diagnostic techniques based on the reactivity. Gel diffusion, complement fixation and latex agglutination are of low reactivity requiring high concentration of antibody and are not useful for the detection of antibody in the first week of infection. Tests of "medium reactivity" are the indirect haemagglutination and indirect immunofluorescence techniques, which can detect antibody during the second week of infection. Tests of high reactivity are the radio-immuno assay and ELISA, which can detect low concentrations of antibody (nanogram or picogram/ml) on the third or fourth day of infection in some cases. ELISA test has been done to detect antibodies using different kinds of antigens
derived from mf or adult worms. One such antigen is Excretory Secretory antigen.

**Immunodiagnosis based on the antigen detection**

Detection of filarial antigens present in the biological fluids such as serum or urine will be more reliable and useful in the early diagnosis of filariasis. These antigens consist of a complex mixture released by different stages of living parasites such as microfilariae, infective larvae and adult filarial worms as well as somatic antigens added to the circulation by the destruction of filarial worms in the host. Franks (1946) for the first time demonstrated the presence of circulating antigen in the sera of filarial patients using the infected sera as antigen in skin test. Dasgupta & Bala (1978), Dasgupta et al (1984) Au et al (1981), Kaliraj et al (1979,1981) observed the presence of antigen in human filarial sera using rabbit anti L.carinii, anti B.phanagi anti-W.bancrofti microfilarial sera and human filarial serum immunoglobulin (FSI)) respectively. Use of IgG fraction of FSI (FSI-G) in sandwich ELISA was found to be quite sensitive in detecting circulating antigen in 27 out of 33 microfilaraemia sera. An apparent positive correlation between the microfilarial density and antigen titre was also observed by Reddy et al (1984). During DEC administration ES antigen titer was maximum at the 4th week due to microfilaricidal effect of DEC (Malhotra and Harinath (1984). Presence of filarial antigen in immune complex was detected by direct ELISA. W.bancrofti mf ES antigen was demonstrated in the immune complex by competitive ELISA (Prasad et al 1983).
Detection of filarial antigen from body fluid

Filarial antigen was also detected in hydrocele fluid from filarial patients. Tanabe (1959) detected the presence of antigen in urine of filarial patients, using rabbit anti *D. immitis* sera. Filarial antigen was also detected in the neat urine samples of microfilaraemia patients by double antibody Sandwich ELISA, using FS IgG and anti rabbit urinary filarial antigen immunoglobulin (Malhotra *et al* 1985) and by radio immunometric assay using $^{125}$I-labelled rabbit IgG antibody to *B. malayi* antigen (Reddy, *et al* 1991). Ramprasad *et al* (1987) fractionated and characterized the urinary filarial antigens (UFA) by gel filtration on Ultrogel ACA-44 followed by SDS-PAGE, to understand the nature of these antigens. Separation and characterization of different antigen fractions from crude antigenic extracts, showed four protein fractions namely UFAC$_1$, UFAC$_2$, UFAC$_3$ and UFAC$_4$. These fractions were tested for filarial antigenicity by Sandwich ELISA. UFAC$_1$ and UFAC$_2$ showed antigenic activity. Zheng Huijun *et al* (1987), developed a Sandwich ELISA to detect circulating parasite antigens in human lymphatic filariasis. They have used a polyclonal rabbit antifilarial antiserum and two monoclonal antibodies namely Mab ES34 and Mab HC11. They could detect more number of circulating parasite antigens with polyclonal antisera. However, one monoclonal antibody ES 34 was found to be useful in antigen detection that correlated with microfilarial density of the night blood. Antigen was also detected in the urine of some microfilaraemic patients. Prasad *et al* (1987) analysed paired serum, urine and filter paper blood specimen for the presence of filarial antigen by immunoradiometric assay. They have demonstrated that urine and filter paper whole blood specimen can be used in conjunction with the immunoassay for the diagnosis of human filariasis.
Dot ELISA

Zheng Hui-Jun et al (1990) designed their study to determine whether the Dot ELISA technique could be used to detect filarial antigens in sera, and to compare the sensitivity of this assay with that of Sandwich ELISA. Monoclonal antibodies and patients sera were used in both tests. In Dot ELISA, 67 of 70 sera from microfilaraemic donors were positive for filarial antigen, where as in sandwich ELISA, all the 64 sera were positive. Dot ELISA was found to be more sensitive than Sandwich ELISA.

DNA probes for detection and identification of human parasites (W.bancrofti, B.malayi)

The identification of nematodes presents problems because of the lack of reliable diagnostic morphological characteristics. However, genomic DNA restriction fragment length differences in repetitive sequence have been used to identify a wide range of nematode species. It was suggested that techniques of molecular characterization could be applied to species and strains of filarial worms, Wuchereria and Brugia in man and mosquitoes. Various candidate probes have now been developed for differentiation and identification of Brugian blood samples (Mc Reynolds et al 1986). Oligonucleotide probes for both Brugia phanagi and B.malayi were developed by Piessens et al (1987) and used to detect single microfilaria in crude preparations of DNA from blood (William et al 1987). In addition, a highly specific and sensitive DNA probe for B. malayi has been proposed as a candidate probe for the diagnosis and detection of infected vectors in filarial endemic areas, during field studies (Rajan 1990).
Summary of different immunodiagnostic tests and various kinds of antigens used in the diagnosis of human filariasis

Based on Antibody detection

Based on Antigen detection

Test for Cell Mediated Immunity

Based on Antibody Detection

Types of Antigens used

1. Immunodiffusion test
   - Human filarial antigen
     - *Wuchereria bancrofti*
     - *Loa loa* (Gentilini et al 1972)
   - Animal filarial antigen
     - *Dipetalonema viteae*
     - *Chandlerella hawking*
     (Crow filarial antigen)
     - *Chatterjee et al 1978*
     - *Setaria digitata*
     (Bright and Raj 1990)

2. Complement fixation test
   - *W. bancrofti*
   - Excretory secretory antigen (Kagan 1963)

3. Counter Immuno electrophoresis
   - *Wuchereria bancrofti* mf antigen
     - Soulable Antigen
       (Kaliraj et al 1977)
     - Whole cell lysate antigen
       (Kaliraj et al 1977)
     - Excretory secretory Ag (mf)
     - *Brugia malayi* mf
       (Dasgupta et al 1984)
       (Das et al 1988)
   - *Dirofilaria immitis*
     (Desowitz and Una 1976)
   - *S. digitata*
     (Sugunam and Keleyaraj 1990)

4. Indirect Hemeaggltination test (IHA)
   - *W. bancrofti* mf antigen
     (Kaliraj et al 1981a)
   - Excretory secretory antigen
   - Filarial serum immunoglobulin
     (Kaliraj et al 1981)
   - *Dirofilaria immitis*
     (Takahashi and Sato 1976)
   - *Breinlia boiliate*
     (Malkit Singh et al 1980)
5. i. Indirect fluorescent antibody test (IFA)
   - *W. bancrofti* mf
     (Gonzaga *et al* 1976)
   - *W. bancrofti* mf fragments
     (Young 1973)
     (Kaliraj *et al* 1979)
   - *Brugia malayi* mf
     (Santos *et al* 1976)
     (Hedge and Ridley 1977)
   - Excretory Secretory
     (Khart *et al* 1983)
   - *Dirofilaria immitis* sliced adult antigen
     (Thomas 1969)
   - *Dipetalonema viteae* egg shell & uterine fluid
     (Ambroise Thomas and Keintrouge 1974)
   - *Brugia malayi* &
     *Breinilia booliati*
     (Sethumadavan *et al* 1988)

ii. Direct fluorescence technique
   - Sheathed mf
     (Ravindran *et al* 1990)

6. Enzyme linked immunosorbent assay
   - Direct ELISA
     Immune complex
     *W. bancrofti* mf antigen
     (Prasad *et al* 1983)
   - Indirect ELISA
     Soluble antigen
     (Kaliraj *et al* 1983)
     Excretory secretory antigen
     (Khart *et al* 1982)
   - Stick ELISA
     (Fila test)
     Excretory secretory antigen
     (Parkhe *et al* 1986)
   - Inhibition ELISA
     Excretory secretory antigen
     (Malhotra and Harinath 1984)

**BASED ON ANTIGEN DETECTION**

1. Circulating filarial antigen (CFA)
   Mf (positive) carrier plasma
   (Kaliraj *et al* 1981)

2. Counter immuno electrophoresis
   Human filarial
   - Rabbit antifilarial sera
     (Kaliraj *et al* 1979)
   - *W. bancrofti* microfilaraemia plasma (FS1g)
     (Kaliraj *et al* 1981)
   - *Brugia malayi* rabbit antisera
     (Weil *et al* 1986)
   Animal filarial
   *D. immitis* rabbit antisera
   (Desowitz and Una 1976)
   (Weil *et al* 1986)
   Antirabbit *L. carinii* sera
   *L. carinii*
   (Dasgupta and Bala 1978)
   Anti *B. phanangi* sera
   *B. phanangi*
   Setaria digitata
   Onchocerca gibsoni
CELLULAR
ASSAY

1. Skin Test
   - *Brugia malayi*
     (Grove et al 1977,
      Chandra et al 1978,
      Katiyar et al 1985
      Murthy et al 1988)
   - *Dirofloria immitis*
     (Sawada Antigen)
     Sawada et al 1969,
     Sawada et al 1965,
     Desowitz et al 1966,
     Sawada et al 1968,
     Sato et al 1969)

2. *In vitro* lymphocyte response
   - *W.bancrofti mf antigen*
     (Metha et al 1980)