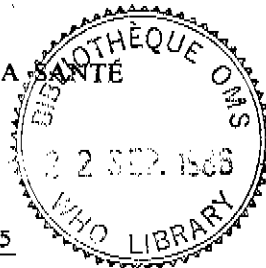




UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR  
RESEARCH AND TRAINING IN TROPICAL DISEASES



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LYMPHATIC PATHOLOGY AND IMMUNOPATHOLOGY IN FILARIASIS:  
REPORT OF THE TWELFTH MEETING OF THE  
SCIENTIFIC WORKING GROUP ON FILARIASIS

*scrotal  
lymphoedema  
P+C*  
*Elephantiasis, Filariasis  
P+C*

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This report contains the collective views of an international group of experts convened by the UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR RESEARCH AND TRAINING IN TROPICAL DISEASES (TDR). It does not necessarily reflect the views of TDR/WHO. In the interests of rapid communication it has been submitted to only minimal editorial revision. Moreover, any geographical designations used in the report do not imply the expression of any opinion whatsoever on the part of TDR or WHO concerning the legal status of any country, territory, city or area or of its authorities concerning the delimitation of its frontiers or boundaries.

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## 1. INTRODUCTION

The twelfth meeting of the Scientific Working Group (SWG) on Filariasis of the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR) was held at Thanjavur Medical College Hospital, Thanjavur, Tamilnadu, India, in cooperation with the Fogarty International Center, Bethesda, MD, USA, which sponsored the attendance of participants from the USA, and with the Indian Council for Medical Research, which sponsored the attendance of a number of observers from India.

The purpose of the meeting was to review the pathogenesis of lymphoedema and other similar clinical manifestations of bancroftian and brugian filariasis. By drawing on the combined knowledge of surgeons, physicians, epidemiologists with field experience of filariasis, lymphologists, pathologists, immunologists with specialized knowledge of the organ systems concerned, and those working on experimental filarial infections in animal models, it was hoped to determine new ways of preventing or treating filarial lymphoedema and what further research is needed.

The opening ceremony was presided over by the Dean of Thanjavur Medical College, Major E.M. Saraswathi, and proceedings were opened by the Director of Medical Education for Tamilnadu State, Dr Lalitha Kameswaran. In her opening address, Dr Kameswaran explained the absence of the Minister for Health and Family Welfare, Dr H.V. Hande, and read a message from him (see Annex I). She drew attention to the importance of filariasis and elephantiasis as a disease problem in India and emphasized the great advances made in the surgical treatment of filarial lymphoedema by Prof S. Jamal and his colleagues at the Clinical Filaria Research Unit of Thanjavur Medical College. She looked forward to the increasing use of medical and health education to help prevent filariasis, and welcomed the news that at least two new filaricidal drugs (ivermectin and CGP 20376) are likely to go into clinical trials in India in the near future.

Dr B.O.L. Duke then replied on behalf of TDR and WHO. On behalf of the Director of TDR, Dr A.O. Lucas, he expressed thanks to the Government of India and to the Government of Tamilnadu State for allowing the meeting to take place in India; to the Dean of Thanjavur Medical College for hosting the meeting; and to the Fogarty International Center and the Indian Council for Medical Research for sponsoring some of the participants. He pointed out that the selection of Thanjavur as the site for the meeting was a reflection of the widespread admiration for the work of Prof Jamal in developing surgical techniques for the relief of filarial lymphoedema. He thanked Prof Jamal and his team of Thanjavur's leading citizens, who had done so much to ensure the

smooth running of the meeting and the arrangements for the participants. It should not be forgotten, he said, that the meeting was taking place at a time when 400 000 people in Tamilnadu State had been rendered homeless by a recent cyclone.

Dr Duke noted that the ultimate aim of the meeting was to study the pathogenesis of filarial lymphoedema so as to develop means for relieving and preventing filarial elephantiasis. Present at the meeting were front-line clinical workers on filariasis from India, distinguished lymphologists with experience in other diseases of the lymphatic system, together with pathologists, immunologists and other researchers working with experimental animal models, who were studying the basic processes involved in the development of disease. It was hoped that this interchange of knowledge would bear fruit, that the laboratory workers would be able to see the human disease problem in the field and that the clinical workers would be made aware of advances in the basic sciences as applied to the lymphatic system.

Finally, Dr Duke pointed out the enormity of the problem of filarial lymphoedema and elephantiasis in India, where at least three million people are already affected to the extent that they can only benefit from surgery and/or physiotherapy.\* It has been calculated that even if 600 surgeons were trained in the skills of Prof Jamal and each surgeon treated 500 cases a year, it would still take ten years to deal with the backlog of cases. It was therefore vital that means should be found to prevent the development of filarial lymphoedema in the coming generation, and this might well be done simply by ensuring that diethylcarbamazine citrate (DEC) treatment is available through the primary health care system for treatment of every person suffering an acute attack of filarial fever and adenolymphangitis.

The Deputy Chairman of the Legislative Council of Tamilnadu, Mr G. Swaminathan, then welcomed the participants to the town, and Prof S. Jamal proposed a vote of thanks to the Director of Medical Education for opening the meeting.

The technical sessions of the meeting were held in the Clinical Society Hall of the Thanjavur Medical College Hospital and were chaired by Dr E.A. Ottesen, Chairman of the Steering Committee of the Filariasis Scientific Working Group.

On 20 November, Prof Jamal gave a demonstration in the operating theatre and in the wards of the techniques of lymphonodo-venous shunt operations, of the physiotherapeutic drainage that is needed after the operation, and of the reconstructive plastic surgery needed to remove surplus tissue. These demonstrations were backed up by video tapes showing actual surgery and physiotherapy.

The next day the participants were taken on a field trip to Vellam village township, a community of some 12 000 persons about 7 km from Thanjavur, where bancroftian filariasis, transmitted by Culex quinquefasciatus, is highly endemic. They were able to see the conditions in which people live, to talk to many individuals suffering from different degrees of filarial lymphoedema and to see the breeding sites of the vector mosquito.

On 22 November, the participants visited a filarial outpatient clinic at Thanjavur Hospital. They were shown how patients are selected for and examined after surgery.

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\* The figure of 14 million is given by the Indian Council for Medical Research Task Force Proceedings, 5-6 May 1980.

## 2. SUMMARY OF DISCUSSIONS

### 2.1 Pathogenesis of Human Lymphatic Filariasis

#### 2.1.1 Clinical aspects

The clinical manifestations of lymphatic filariasis associated with adult or developing adult worms are characterized by acute adenolymphangitis, usually accompanied by fever, and by chronic obstructive lesions that develop years later, often after repeated acute attacks. These manifestations have been well described in brugian and bancroftian filariasis, but the mechanisms underlying them are poorly understood. In addition, the exact course of disease development in lymphatic filariasis is not fully understood. It is not known whether clinical manifestations are always preceded by an asymptomatic microfilaraemic stage. Indeed, the terms "microfilaraemia" and "amicrofilaraemia" need to be used with caution. A patient who is amicrofilaraemic on a 20 mm<sup>3</sup> blood film may well be revealed as microfilaraemic if 1-3 ml of blood is examined by Nucleopore filtration. Furthermore, it can never be entirely excluded that amicrofilaraemics (assessed on 1-3 ml blood filtration) may harbour in their lung capillaries a reservoir of microfilariae, which are not released into the peripheral blood.

Differences in the anatomical distribution of the clinical manifestations of brugian and bancroftian filariasis may be due in part to different tropisms of the parasites for particular anatomical locations, which might govern the location of the adult worms in the human lymphatics. However, pathological evidence supporting these presumptions is currently inadequate.

Clinical differentiation between lymphoedema and elephantiasis is often difficult and the two are frequently grouped together. For clinical trials, however, it is necessary to adopt a uniform scheme of classification for lymphoedema. The classification given below is similar to that adopted by the International Society of Lymphology at its meeting in Adelaide, Australia, in 1985, but its use in filariasis has not yet been tested.

Grade I lymphoedema: mostly pitting oedema; some fibrosis; spontaneously reversible on elevation.

Grade II lymphoedema: mostly non-pitting oedema; much fibrosis; not spontaneously reversible on elevation.

The adjectives "mild", "moderate" and "severe" can be applied to these categories.

Grade III lymphoedema (elephantiasis): a monstrous increase in volume in a Grade II lymphoedema, with dermatosclerosis and papillomatous outgrowths.

The WHO classification of disability is recommended when classification on this basis is necessary (e.g. in clinical trials).\*

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\* International Classification of Impairments, Disabilities, and Handicaps: A Manual of Classification Relating to the Consequences of Disease. Geneva: World Health Organization, 1980, 207 pp. Sw. Fr. 15. (English and French). This publication is available through the WHO network of designated sales agents (listed in all WHO publications) or may be obtained directly from the World Health Organization, Distribution and Sales Service, 1211 Geneva 27, Switzerland.

The three most compelling clinical issues that need to be investigated in lymphatic filarial disease are: the aetiology of the acute episodes of adenolymphangitis with fever, the risk factors that govern the acquisition of any form of the disease and the factors that govern the progression of the disease to its chronic form. Currently, our knowledge in these areas is meagre: the problems could best be addressed by using a combination of clinical, epidemiological, microbiological and parasitological techniques.

It is generally assumed that in bancroftian or brugian filariasis the microfilariae are in no way responsible for the obstructive lesions in the lymphatics and that the microfilariae delivered by fertile female worms in the lymphatics find their way into the blood by passing with the flow of lymph through the thoracic duct. There is no direct evidence, however, for either of these assumptions. Indeed, microfilariae may enter the blood by penetrating the walls of the post-capillary venules in the lymph nodes, thus possibly contributing to lymphatic pathology. The route by which microfilariae enter the blood could be readily determined from work on animal models, and such experiments should be undertaken.

The obstructive and other lesions in the lymphatics and lymph nodes which lead to lymphoedema in filariasis are attributed to developing and adult worms (i.e. the L<sub>3</sub> and L<sub>4</sub> stages), which are relatively short-lived but which both undergo a moult, and to the young adult stage developing up to and including the fertile adult male and female worms. Evidence from animal models, especially the nude mouse, suggests that simple physical blockage by adult filariae is not the cause of the lymphoedema, but that some factor(s) produced by the living worms (and/or possibly the by-products of the L<sub>3</sub>-L<sub>4</sub> and L<sub>4</sub>-young adult moults), coupled perhaps with interaction with the host's humoro-cellular inflammatory response, may be responsible for lymph drainage malfunction.

#### Recommendations

To increase our understanding of the pathogenesis of the clinical aspects of lymphatic filariasis, the following recommendations are proposed:

- (i) Long-term clinical and parasitological studies are needed to understand the course of infection of lymphatic filariasis in endemic communities, as well as among uninfected people migrating to areas where filariasis is endemic. Such studies should include information on environmental factors (e.g. seasonal variation in transmission and regions of the body bitten preferentially by the vector), other contributing factors (e.g. physical exertion and nutritional status); and sociological aspects (e.g. socioeconomic status, use of footwear, sleeping habits, etc., in relation to exposure to mosquito bites).
- (ii) To understand the pathogenesis of the adenolymphangitis of filariasis, studies are required to assess: the role of possible bacterial infection; patterns of microfilaraemia and the levels of parasite-derived products during and between acute episodes; the cells involved in parasite destruction or their constituent inflammatory products (e.g. macrophages, eosinophils and eosinophil-derived products); and host responses to L<sub>3</sub> antigens during such episodes.
- (iii) Examination of the role of circulating antigens, localized parasite products, immune complexes, products of T-cells and macrophages, etc., as the effector arm of recurrent adenolymphangitis should also be encouraged.

### 2.1.2 Lymphological aspects

Although details of the causes of the pathological conditions seen in filariasis are largely unknown, studies based on the interruption of lymphatic flow in other nonfilarial human diseases, as well as those in animal models of lymphoedema, have indicated that the clinical spectrum of chronic filariasis can be explained in part by the high protein nature of the fluid accumulating in the tissues. Furthermore, the distribution of the lymphoedema (e.g. distal extremity versus the entire extremity) might be explained by the presumed location of the lymphatic obstruction.

The lymphatics need not be physically obstructed in lymphoedema; many factors can cause the collecting lymphatics either to go into spasm or to become widely dilated in flaccid paralysis. There is also variation between individuals in the number of the lymphatics and their anatomical arrangements, the number and activities of the proteolytic macrophages, and the resistance of the tissues to oedema.

Experimental work has indicated that changes in the architecture of the lymphatic endothelium may play an important role in the pathogenesis of lymphoedema. Also, from preliminary observations it would appear that the immunocompetent cells in the lymphatics have structural and functional characteristics different from those of similar cells in peripheral blood. An understanding of the importance of such differences is critical to the study of the pathogenesis of lymphatic filariasis, since all cellular studies conducted so far have been confined to the peripheral blood.

### Recommendations

- (i) Lymphoscintigraphic studies should be used to measure lymphatic function and to determine the level of obstruction in the various forms of lymphatic filariasis, such as limb oedema, hydrocoele or chyluria. The same technique may be used to explain the differences in common clinical expression of brugian filariasis (involvement of the leg below the knee; no genital disease) and bancroftian filariasis (disease often involving the entire limb; genital disease common, especially in males). Lymphoscintigraphic studies of individuals with asymptomatic infection should be included.
- (ii) The use of simple noninvasive procedures (e.g. inspection, palpation and skin-fold measurement) is recommended in order to identify individuals with abnormal lymph absorption syndromes in endemic communities. These individuals should subsequently be examined by tonometry and lymphoscintigraphy to determine the expression of the developing disease.
- (iii) Studies on the constituents of lymphatic fluid in patients undergoing surgery for lymphatic obstruction are recommended, since they would help resolve the issue of the existence of high protein oedema in the "low-output" failure of filarial lymphoedema and would provide valuable source material for immunological and pathological studies, so that both the compartmentalized immune response and the possible lymphangiogenic and fibrogenic factors could be examined directly.
- (iv) Training should be provided in clinical lymphological techniques to permit early detection of lymphatic changes.
- (v) WHO should encourage universities to teach the elements of basic and clinical lymphology, so as to enable graduates to diagnose lymphoedema by inspection and palpation.

### 2.1.3 Immunogenetic aspects

Differential susceptibilities to infection, and thereby differential immune responses among persons with filariasis, are manifested in differing clinical presentations. Thus, the genetic background of the endemic population should be considered, particularly since filarial and other parasite antigens must be presented to the immune response in a fashion restricted by major histocompatibility complex (MHC) products. The only two studies which addressed this issue directly among endemic populations used differing experimental protocols and produced conflicting results. There is a need to define the genetic bases for the varied immune responses seen in endemic populations.

#### Recommendations

Family- and population-based studies should be carried out to define:

- (i) The genetic background of patients with different clinical manifestations of filariasis (including chyluria), with particular reference to: HLA-A, -B, -C typing; HLA-Dr and minor immune response loci; complement polymorphism; and other markers of genetic susceptibility.
- (ii) The ability of each population to respond to externally administered vaccines, novel antigens and recall antigens.

### 2.1.4 Modulation of the immune response

The wide variability of clinical pathology seen among those living in regions endemic for the lymphatic forms of filariasis is thought to reflect the immunological responsiveness of the human host. However, much of the information on which this hypothesis is based comes from isolated cross-sectional studies from regions that differ both in the causal parasite and in the clinical manifestations of the disease. Comparative immunological studies across regional and parasitological boundaries are therefore required.

Virtually all studies examining the immunological responsiveness (both humoral and cell-mediated) of patients with filarial infections have used crude extracts of parasite material containing hundreds of proteins and glycoproteins. The particular antigenic determinants in these extracts responsible for triggering the immune response in vitro or in vivo are unknown. Thus, until defined antigen preparations are available, the nature of the immune response in filariasis will remain ill-defined and nonspecific.

Data have accumulated on factors that modulate the immune response to crude filarial antigens in vitro. However, these studies have focused on patients without lymphatic obstruction, and investigations need to be undertaken on patients with adenolymphangitis and elephantiasis who characteristically respond to parasite antigens, in order to examine how such a response may be modulated.

Since immune complexes and circulating parasite antigens have been identified in experimental and human filarial infections, their effects must be taken into account along with other regulatory factors, such as anti-idiotypic antibodies or prostaglandins.

Questions that need to be addressed include the following:

- (a) Can the role of immune complexes and circulating parasite antigens be elucidated with regard to immunoregulation?

(b) Can the network theory be applied to the regulation of the immune response in lymphatic filariasis?

(c) Can antigen-specific B- and T-cell cloning be used to examine immunomodulation?

(d) Will the cloning of either the circulating antigens or the molecules responsible for altering the host immune responses to filarial parasites provide useful tools for modulating the immune response and thus alter the subsequent pathological reactions?

#### Recommendations

- (i) The use of methods to analyse qualitatively those antigens responsible for inducing both immune responses and immune tolerance to filarial parasites should be encouraged.
- (ii) Highly defined or molecularly cloned parasite antigens should be used to obtain a clearer picture of the host immune response.

#### 2.1.5 Parasitological aspects

While the host response plays a role in determining the pathology of lymphatic filariasis, the parasite itself must also be implicated, at least in part, in determining the varied pathology seen in human filarial infections. Although there are clear-cut morphological distinctions among the three filarial parasites responsible for chronic lymphatic obstruction, the overlapping of clinical syndromes between bancroftian and brugian filariasis and the difference in presentation within areas imply basic parasitological differences, which may extend to different forms or strains within each species complex. The fact that certain parasites seem to migrate to particular sites in the human body and thus alter the expression of the clinical disease requires investigation. In addition, the anatomical location of the parasites in patients from most areas endemic for lymphatic filariasis has never been adequately defined either in the human host or in animal models.

Another factor which needs to be considered in relation to the location of adult worms in the body is the influence which the preferred biting site of the different mosquito vectors may have on the site of inoculation of infective larvae.

The possibility that bouts of filarial fever and acute adenolymphangitis are chronologically related to the previous input of batches of infective larvae also needs to be considered.

#### Recommendations

- (i) Characterization is needed of the genetic and antigenic differences among isolates of the same species and between species. Are these differences responsible for the varied clinical conditions seen?
- (ii) Complete post-mortem examinations are imperative in patients with bancroftian and brugian filariasis, with and without lymphoedema or elephantiasis, in order to determine the functional and anatomical localization of adult parasites and microfilariae, as well as the pathology (in situ) associated with their deposition. Pathologists with experience of the lymphatic system should be recruited to areas endemic for filariasis so that these issues can be examined.

### 2.1.6 Environmental aspects

While "environmental" factors may be less likely than genetic factors to determine how the host responds to the filarial parasite, they need to be examined, particularly in the light of work done on in utero sensitization and on nutritional factors affecting the immune response. Examination of environmental factors acting during the perinatal and neonatal periods, with long-term follow-up when appropriate, should be encouraged, such as:

- (a) cellular and antigen studies on cord blood;
- (b) the possibility of immune tolerance developing to filarial antigens in infants of infected mothers;
- (c) breast milk antigen/antibody studies;
- (d) the acquisition of parasite-specific immune responses over time.

Studies of other factors, such as nutritional, environmental and socio-logical conditions, as they relate to the immune response to filarial parasites, could also be of great value.

## 2.2 Treatment and Prevention of Filarial Lymphoedema: Treatment of Chronic Lymphatic Obstruction in Filariasis

### 2.2.1 General

The treatment of lymphatic disease associated with Wuchereria bancrofti, Brugia malayi or B. timori infection in human subjects represents a major therapeutic problem in endemic areas of Africa, Asia and the Pacific region. There is currently no uniform approach to the management of the chronic disease manifestations of lymphatic filariasis, which include hydrocoele, lymphoedema and elephantiasis of an extremity, lymphatic inflammation/obstruction of the scrotum and its contents (epididymis, spermatic cord, testicles), and chyluria.

It is highly encouraging that there are now several treatment methods -- physiotherapeutic, chemotherapeutic and surgical -- which hold promise for reversing the signs of chronic lymphatic obstruction previously thought to be irreversible. The optimal modes of therapy for the various disease manifestations remain unclear. The various methods of treatment that may be useful for managing each of these are summarized below and recommendations for research needed to define the optimal method(s) of treatment are presented.

The question of whether the live or the dead adult worms cause more damage to host lymphatic drainage is vital when filaricidal therapy with DEC or other drugs is considered. In general, from results of work on animal models (including the nude mouse) and from experience with DEC chemotherapy in human subjects, it appears that macrofilaricidal treatment has a beneficial rather than a detrimental effect on acute adenolymphangitis and lymphoedema. This is certainly true in the early stages of infection, but in the later stages, when gross obstructive damage has led to fibrotic lymphoedema and elephantiasis, macrofilaricidal treatment may be much less helpful. Treatment then depends primarily on re-establishing lymphatic drainage by surgical and physiotherapeutic means.

The best working hypothesis would appear to be that macrofilaricidal treatment (currently with DEC as the only drug available) should be undertaken as early and as often as acute or "acute-on-chronic" clinical manifestations occur. This approach appears more likely to prevent or delay the onset of

severe lymphoedema than a "non-filaricidal" policy based on the belief that dead or dying adult worms are the cause of lymphatic malfunction.

### 2.2.2 Hydrocoele

#### (a) Definition and pathophysiology

This condition, extremely common in bancroftian filariasis, manifests clinically as a swelling of the reflection of the peritoneal lining that surrounds each of the testicles. Clear hydrocoele fluid accumulates in this closed sac as a result of lymphatic blockage in draining lymphatics located in the retroperitoneal and subdiaphragmatic areas. Hydrocoele is the most common disease manifestation of W. bancrofti infection but has not been recorded in Brugia sp. infections where W. bancrofti was not also present.

#### (b) Treatment

Excision or eversion of the hydrocoele sac, with or without drainage, is currently the most common method of therapy. In male subjects with small hydrocoeles (less than 50 ml fluid), sclerosing agents such as tetracycline may be injected locally with some benefit. All subjects with hydrocoeles in filarial endemic areas should receive treatment with DEC.

#### (c) Recommendations

- (i) The optimal surgical procedure for management of filarial hydrocoele needs to be defined.
- (ii) The possible usefulness of DEC therapy in the management of filarial hydrocoele needs to be examined, particularly in areas where surgery is unavailable.

### 2.2.3 Scrotal lymphoedema and lymphatic inflammation/obstruction of scrotal contents (epididymis, spermatic cord, testis)

#### (a) Definitions and pathophysiology

These conditions include fluid collection in the testis, epididymitis, funiculitis (swelling of spermatic cord) and thickening of the scrotal skin. In female subjects, no homologous lesions have been reported involving the ovary or fallopian tubes, although lymphoedema of the vulva may occur.

#### (b) Treatment

During the acute phase of a filarial "attack" on these organs, a conventional course of DEC should be given, followed by periodic administration to prevent recurrent attacks. This approach may be helpful in subjects without long-standing or severe disease. However, most subjects present with severe disease that has been left untreated for a long time. In this situation, current experience in India suggests that DEC may not reduce the swelling, although it may reduce the frequency of painful febrile episodes. Surgical approaches, such as lymphovenous drainage in the inguinal region, are then performed with variable degrees of success.

#### (c) Recommendations

- (i) The utility of various DEC regimens in the reduction of genital lymphoedema should be investigated.
- (ii) Standard surgical procedures for drainage should be established.

## 2.2.4 Lymphoedema and elephantiasis of the extremities

### (a) Definition and pathophysiology

Recurrent episodes of limb lymphoedema, resulting first in pitting oedema and then in non-pitting oedema with loss of skin elasticity and fibrosis, are the result of anatomical and/or functional blockage of the lymphatics. The legs are more commonly affected than the arms.

In W. bancrofti endemic areas, leg swelling may involve the thigh as well as the lower leg, while in B. malayi infection only the portion of the leg below the knee appears to be swollen. Secondary infections of the skin (bacterial and fungal) are common in such subjects, particularly with those who do not wear shoes.

Elephantiasis is often remarkably well tolerated by the patient, provided it is not excessive and there is no secondary infection or malodorous lymphorrhoea.

### (b) Treatment

Studies in areas of Indonesia where B. timori infections exist indicate that DEC administered over a long period of time (more than one year) can result in regression of lymphadenopathy and, more strikingly, complete disappearance or significant regression of elephantiasis. Work on these lines should be continued, as should the current trials of benzopyrones as a means of reducing lymphoedema, presumably by stimulating macrophages to remove protein and produce collagenase that will dissolve fibrosis.

Similar observations using "high" doses of DEC (i.e. a total dosage of 3.0 to 4.2 g given over 3 to 7 days in W. bancrofti infections or 1.5 to 2.0 g given over 3 to 10 days in B. malayi infections) have been made in Chinese subjects. They suggest that this drug may be beneficial, especially when used in conjunction with compressive dressings. Heat treatment and bandaging of the extremity, as well as injection of mulberry leaf extract, have also been reported to be beneficial in China.

Striking beneficial responses, with regression of elephantiasis, have also been observed with lymphovenous drainage procedures, followed by adequate postural drainage and physiotherapy and, if necessary, the removal of excess subcutaneous fatty and fibrous tissue in the lower extremities. As in all surgical procedures for filariasis, this is accompanied by DEC therapy. Although the shunt operation can be carried out under local anaesthesia, the physiotherapeutic after-care and any subsequent operations to remove excess tissue require full hospital surgical facilities.

Finally, it should be emphasized that antibacterial or antifungal agents should be administered when these secondary infections occur. The specific antibiotic to be used depends on the organisms involved.

### (c) Recommendations

- (i) The efficacy of DEC in reversing grade II lymphoedema, with or without elephantiasis (bancroftian and brugian), needs to be studied in a controlled fashion. The optimal drug dose should be established.
- (ii) The possible utility of surgical approaches, such as lymphovenous drainage, in large populations needs to be investigated. Studies should consider the feasibility of this surgery in regional centres where resources are limited and of the use of lymphatic massage and other physical techniques to reduce the time spent in hospital before surgery.

- (iii) It should be ascertained whether complex decongestive physiotherapy, which has proved highly effective in all forms of lymphoedema that occur in the developed world, is equally effective in lymphoedema due to filariasis. If so, the training of physiotherapists to treat this condition should be encouraged.
- (iv) Efforts should be made to promote the prevention of injuries to the feet in cases of grade I lymphoedema of the lower limb (e.g. by providing low-cost shoes) and to improve the recognition of early mycotic infection and erisypelas.
- (v) The possible efficacy of benzopyrones in regression of animal and human filarial lymphoedema should be examined.
- (vi) The possible role of steroids and anti-inflammatory agents in reversing lymphoedema should be examined.
- (vii) The mechanism of action of DEC in reversing lymphoedema in human subjects and in experimental animal models needs to be defined.
- (viii) The effect of DEC and other agents on the course and frequency of attacks of filarial fever and adenolymphangitis in infected subjects living in endemic areas needs to be investigated in order to assess the influence of such treatment, repeated with each attack, on the development of chronic lymphoedema and on the frequency with which acute episodes recur.
- (ix) Controlled clinical trials should be undertaken to assess the effects of the Chinese methods of treatment for lymphoedema: (a) dry heat and (b) mulberry leaf extract.
- (x) The active pharmacological principle(s) in mulberry leaf extract, used in the treatment of elephantiasis, should be investigated and its mode of action defined.

#### 2.2.5 Chyluria

##### (a) Definition and pathophysiology

Chyluria may be defined as the excretion of chyle in the urinary tract. A minority of affected subjects may also have haematuria. The basic pathophysiology is related to blockage of the retroperitoneal lymph nodes below the cisterna chyli, with consequent efflux and flow of the intestinal lymphatics directly into the renal lymphatics. The large amount of chyle in this site is then passed into the urinary tract, producing a "milky" urine, which contains considerable quantities of foodstuffs originating from the gastrointestinal tract. The condition is painless, but large amounts of dietary lipids, proteins and possibly fat-soluble vitamins are excreted, leading to weight loss or even inanition.

##### (b) Treatment

Treatment is currently based on the clinical history of the individual patient. In subjects with short-term (less than six months) low-grade chyluria, conservative approaches using DEC therapy and restriction of dietary fats may be helpful. In the majority of subjects, in whom chyluria continues despite these measures, surgical approaches are indicated. These include disconnection of the renal hilar lymphatics by nephropexy. Surgical procedures to be avoided include nephrectomy, renal capsular decortication and periureteric disconnection.

Instillation of silver nitrate into the renal pelvis also induces sclerosis and has been reported to "cure" chyluria in some endemic areas. However, as with all sclerosing agents, its use is not without danger.

(c) Recommendations

- (i) The exact sites of blockage in the lymphatics in chyluria need to be better defined.
- (ii) The effectiveness of DEC and dietary restriction in altering the amount of urinary fat excretion should be investigated quantitatively.
- (iii) Long-term follow-up of surgical lymphatic drainage procedures is needed.
- (iv) The effects of chyluria on fat and protein metabolism and on lipid-soluble vitamin balance need to be investigated in endemic areas where clinical research centres are available.
- (v) The prevalence of chyluria in various endemic areas needs to be determined.

2.3 Animal Models of Filariasis and Their Relevance to the Pathogenesis and Treatment of Human Disease

2.3.1 General

Existing animal models are probably sufficient for detailed clarification of the pathogenesis of human lymphatic filariasis. Currently available models include: Mongolian jird/Brugia spp.; nude mouse/Brugia spp.; ferrets/B. malayi; dogs and cats/Brugia spp.; and leaf monkeys/B. malayi, W. bancrofti and W. kalimantani. In all these systems, gross and histological changes seen in lymphatics are quantitatively similar to those described in human infections with B. malayi and W. bancrofti.

2.3.2 Models for acute and chronic filarial disease

Lymphatic diseases and the lesions associated with filarial infections may be either acute or chronic.

2.3.2.1 Acute filarial disease

Acute human filarial disease is characterized by recurrent episodes of lymphadenitis and lymphangitis, accompanied by systemic symptoms of fever and malaise. It is the most common presentation of filarial infection and is a serious cause of morbidity in endemic regions. Even so, its pathogenesis remains undefined and little work on this topic has been performed in animal models. More emphasis should be placed on such research.

The systems and experimental designs used should be capable of defining and characterizing the causal host and parasite factors responsible for acute filariasis. There is some evidence that episodes of transient lymphadenitis, lymphangitis and lymphoedema occur in the Brugia spp./cat or dog model systems. Nude mice reconstituted with splenic lymphocytes from heterozygous littermates also show an acute lymphangitis and should be used to study the condition. It would also be useful to study acute reactions in animals with different immune states: i.e. those made resistant by the use of irradiated infective larvae; those immunized, but not made resistant, by passive sensitization with antigen; and nonimmune individuals.

The introduction of specific parasite factors into the lymphatic system of experimental animals would be useful. Potential candidates for injection include both soluble and particulate parasite products related to the periodic production of microfilariae, as well as reproductive products, larvae and culture fluids of larvae or adult worms. Sampling of lymph from the lymphatics of injected animals, before and after induction of reactions to parasite factors, would be useful.

#### Recommendation

- (1) Animal models should be used for research into the pathogenesis of acute filarial disease.

#### 2.3.2.2 Chronic obstructive lymphatic disease

Lymphoedema and resulting elephantiasis are considered to be the consequences of obstructive inflammatory reactions induced by filarial infections. The sites of these obstructive lesions, which may occur with or without accompanying lymphoedema, should be studied and compared with obstructions that occur in nonfilarial lymphoedema. In dogs and rats which have undergone artificial ligation of the lymphatics together with certain ancillary interventions, there is first a short-lived oedema, which then clears and is followed, only after a year or more, by chronic oedema. During the latent phase there may be striking lymphographic and histopathological changes despite the lack of oedema. These findings indicate that a very long follow-up is often needed to detect the development of chronic clinical lymphoedema.

Chronic obstructive disease, and the host and parasite factors involved in its development, can be considered in two stages: (i) mechanisms associated with the induction, maintenance and regulation of the lymphatic inflammatory response; and (ii) factors and physiological conditions associated with filarial lymphoedema which have shared features with nonfilarial lymphoedema. Significant initial data exist on these states in several animal model systems, but some models are more suitable than others for particular stages of disease development, owing to their lower costs and the availability of preliminary data. The rodent systems (Mongolian jird/Brugia spp. and nude mouse/Brugia spp.) are particularly well suited for detailed studies of initial lesion formation and regulation. These studies should be expanded to include characterization of the host and parasite factors involved in lesion genesis, including the effect on subsequent lesion development of in utero and/or neonatal exposure to parasite factors. Studies of lymphatic function in chronic obstructive disease should employ techniques which do not further damage affected lymphatics.

The pathogenesis of filarial lymphoedema has been less well studied. However, several models are suitable for this purpose, including the Brugia spp./dog or cat systems; the B. malayi/ferret model system, only recently described; and the B. malayi/nude mouse system. Of particular interest is the further investigation of the B. malayi/ferret model for obstructive lymphoedema and elephantiasis in a permissive host. Studies should concentrate on physiological mechanisms of lymphatic dysfunction that are distinctive for filariasis and yet common to obstructive lymphoedema. Contemporary lymphological methods should be used but attempts should also be made to develop new quantitative methods. Further basic information on the workings of the obstructed (filarial and nonfilarial) lymphatic system is needed. An animal model for filarial chyluria should also be sought. Experiments conducted in vivo and in vitro should be encouraged to study the apparent angiogenic and fibrogenic stimuli associated with filarial lymphoedema, as well as the participation of endothelial cells and lymphocytes.

In consultation with scientists experienced in defining functional and mechanical lesions of the lymphatic system, the features of morbid anatomical studies in animal model systems should be compared closely with those of detailed autopsies of patients with elephantiasis.

Research on the development of B. malayi and W. bancrofti lesions in human subjects should examine the role which the site of vector feeding may have on subsequent lesion development. Studies on the site of infection and on possible regional lymph tropisms of filariae should be conducted. Useful models for this study would include Brugia spp. in cats, dogs, jirds and monkeys.

#### Recommendation

- (i) Animal models should be used to study the pathogenesis and treatment of filarial lymphoedema.

#### 2.3.3 Recently discovered, specialized models

##### 2.3.3.1 The leaf monkey (Presbytis spp.)/W. bancrofti and W. kalimantani model

The use of W. kalimantani and W. bancrofti in Presbytis spp. should be continued and further encouraged. This would be more practicable in areas where leaf monkeys are found naturally, for there are many difficulties with their exportation and maintenance.

The prepatent interval in this infection is between 5 and 11 months, most commonly 7 to 8 months, and the best laboratory vector has been found to be Aedes togoi.

The histopathological lesions seen in the W. bancrofti/Presbytis spp. model are similar to those in human bancroftian filariasis. Studies using this model should give insights into the pathogenesis of human disease. The potential of the W. kalimantani/Presbytis spp. model has not yet been fully realized, and workers in endemic areas should be encouraged to study the induction of acute filarial disease in this model, together with experimental chemotherapeutic studies.

#### Recommendation

- (i) The Presbytis/Wuchereria model should be used to investigate the action of new filaricides and to study the pathogenesis of filarial disease.

##### 2.3.3.2 The nude mouse as a new model for pathogenesis of filarial lymphatic disease

The congenitally athymic and immunodeficient nude (nu/nu) mouse/B. malayi model of lymphatic filariasis is a valuable new system which provides several advantages over current models, and further research using filarial parasites in the nude mouse should be encouraged. Thus far, B. malayi appears to be significantly more pathogenic in the mouse than B. patei or B. pahangi, a difference perhaps masked in conventional models by host immune responses to the parasite.

The nude trait is available on a wide variety of inbred strain backgrounds, the mouse is immunologically well-defined and immunological reagents are readily available. Furthermore, the ability to manipulate the nude animal's immunological responses to parasite antigens allows character-

ization of protective responses to infective larvae, as well as those responses involved in the pathogenesis of filarial lesions and worm killing. Work to date has shown that clinical signs of B. malayi infection in nude mice resemble those seen in human subjects, with dilatation and tortuosity of parasitized lymphatics, lymphoedema of limbs and skin changes.

Immunological reconstitution of nude mice, chronically parasitized by B. malayi, kills adult worms and produces lymphatic pathology which closely resembles that seen in human infection, but this is followed by clinical resolution of the lymphoedema and skin changes. Dilated subcutaneous lymphatics can readily be cannulated in infected nude mice and physiological measurements using modern lymphological techniques can be made.

The immunodeficient status of the nude mouse allows examination of the direct effects of the parasite and its metabolic products on the development of filarial lesions. Nude mice harbouring adult B. malayi have massively dilated lymphatics, which provide a valuable source of large quantities of lymph containing potentially active metabolic products and excretory-secretory (ES) antigens produced by worms living in a physiological environment. The analysis and characterization of these products, which are free of products of the host's immune response, are simpler than with conventional hosts. Examination of the direct effects of purified worm products on lymphatics of animals in vitro and on lymphatic explants of cells in vitro is recommended. The effects of DEC and of macrofilaricidal arsenicals on adult B. malayi worms in nude mice need investigation and the effects of haemolytic streptococcal infection in producing lymphoedema in these animals should be studied. Comparison with normal mice should also be made and the possibility explored of infecting with B. malayi other strains of mice which have different cellular immunological deficiencies.

The nude mouse/B. malayi model should also be investigated as a potential screen for micro- and macrofilaricidal drugs and for detailed examination of the mechanisms of drug action. Drug action studies would be facilitated by comparison of drug activity and clearance in immunodeficient mice with drug activity in immunocompetent conventional models. Large numbers of small, inbred animals harbouring reproducible B. malayi infections would provide consistent and reproducible results. For this purpose, the establishment is recommended of colonies of nude mice parasitized with B. malayi or other relevant filariids for the purpose of drug studies.

Other important filariid species which develop in man or animals should be screened systematically for their ability to develop in nude mice. The nude mouse might allow the development of Onchocerca volvulus, for which there is currently no suitable animal model, thus providing an opportunity for detailed characterization of the parasite, study of larval tropisms and of pathogenicity in the host, and investigations into its susceptibility to chemotherapeutic agents.

#### Recommendations

- (i) The nude mouse model should be used to study the pathogenesis of filarial lymphatic obstruction, especially the endothelial cell proliferative response induced by the parasite and the inflammatory obliterative reaction induced by the transfer of immunocompetent lymphocytes to these immunodeficient animals.
- (ii) The model should also be developed for its potential in chemotherapeutic screening and for its ability to support the development of O. volvulus and other human filarial parasites.

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DUKE, Dr B.O.L., Secretary, Scientific Working Group on Filariasis

4. LIST OF WORKING PAPERS

- |                   |  |
|-------------------|--|
| Campbell, J.R.    | Immunology of bancroftian filariasis in the leaf monkey<br>( <u>Presbytis cristata</u> ) |
| Casley-Smith J.R. | Tissue changes in high-protein oedemas   |
| Connor, D.H.      | Pathology of filarial elephantiasis and hydrocoele                                       |
| Dissanaike, A.S.  | The life-cycle of lymphatic filarial parasites   |
| Ewert, A.         | Experimental lymphatic dysfunction caused by <u>Brugia malayi</u>                        |
| Földi, M.         | Classification of lymphoedema and elephantiasis  |
| Hammerberg, B.    | Pathogenesis of limb oedema in <u>Brugia</u> -infected dogs                              |
| Jamal, S.         | Indications and success of a surgical approach to<br>filarial elephantiasis              |
| Kar, S.K.         | Lymphatic nodules in human filariasis  |

- Kazura, J.W. and Forsyth, K. Relationship of microfilaraemic status to lymphatic disease in Papua New Guinea
- Klei, T.R. Pathogenesis of lymphatic lesions in Brugia pahangi-infected jirds
- Kumaraswami, V. Clinical aspects of bancroftian filariasis: An appraisal of some issues
- Mak, Joon Wah Lymphatic and splenic pathology of Brugia malayi infections in the leaf monkey (Presbytis spp.)
- Nutman, T.B. Immunoregulation in man and its relationship to lymphatic pathology in filariasis
- Olszewski, W.L. Lymphatic obstruction: Physiological and therapeutic aspects
- Partono, F. Clinical differences in the lymphatic manifestations of bancroftian and brugian filariasis
- Piessens, W.F., Kurniawan, L. and Koiman, I. Host immune reactions during the early stages of infection with Brugia malayi
- Tripathi, V.N.P. Pathophysiology and treatment of filarial chyluria
- Vickery, A.C., Albertine, K.H. and Nayar, J.K. Observations of lymphatic pathology in nude mice parasitized by Brugia spp.
- Witte, M. and Witte, C.W. Physiology and pathophysiology of the lymph circulation in man, with a note on lymphangiogenesis and lymphological syndromes
- Zheng Huijun Methods of treatment for filarial elephantiasis used in China

ANNEX I

OPENING MESSAGE FROM DR H.V. HANDE  
MINISTER FOR HEALTH AND FAMILY WELFARE FOR  
THE STATE OF TAMILNADU, INDIA

I am glad to learn that distinguished scientists from various parts of the world have devoted their time and energy to the study of a disease -- namely filariasis -- which has affected hundreds of thousands of people in several tropical countries. Knowing the importance of the disease and its affliction of the poor, the Government, under the able and far-sighted leadership of the Chief Minister, the Honourable Dr M.G. Ramachandran, established in 1981 the Clinical Filaria Research Unit at Thanjavur Medical College, the venue for this Scientific Working Group meeting.

We are grateful to the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR) for devoting the twelfth meeting of its Scientific Working Group on Filariasis to lymphatic pathology and immunopathology in filariasis; to the Fogarty International Center for supporting the attendance of specialists from the United States of America; and to the Indian Council for Medical Research for supporting the attendance of our national experts. I hope the five-day deliberations will help to provide cheaper and more effective methods for the prevention and treatment of lymphatic filariasis.

ANNEX II

SUMMARIES OF WORKING PAPERS

Most participants presented working papers at the meeting. Each presentation was followed by a discussion. Summaries of these papers are given below, under three main headings: anatomy and physiology of the lymphatic system; clinical and pathological aspects of filarial infection in man; pathology and immunopathology in experimental animals.

ANATOMY AND PHYSIOLOGY OF THE LYMPHATIC SYSTEM

Physiology and Pathophysiology of the Lymph Circulation in Man, with a Note on Lymphangiogenesis and Lymphological Syndromes  
Marlys H. Witte and C.W. Witte

Two concepts were introduced to put filariasis into the perspective of the broad discipline of lymphology (the study of the integrated workings of lymphatics, lymph nodes, lymph and lymphocytes in health and disease):

(a) A unifying concept termed the "lymph imbalance theory" views oedema or effusion as a disturbance in the circulation of extracellular fluid, where lymph formation exceeds lymph absorption. In some instances, the underlying nature of the disturbance (i.e. whether high- or low-output failure of lymph flow) is readily ascertained by simple examination. At other times, it may require sophisticated measurements of the forces on both sides of the microvascular barrier, consideration of microvascular permeability and surface area, examination of organs distant from the oedematous site, or assessment of the capacity of tissue fluid or lymphatic drainage. Once the nature of the imbalance is understood, appropriate treatment is devised to reduce lymph formation or enhance absorption, and this is then evaluated in terms of restoring the balance.

(b) Lymphoedema, lymphangiectasia, lymphatic tumour formation (lymphangioma and lymphangiosarcoma) and lymph nodal dysfunction are generally classified as separate entities. Yet two or more of these phenomena may coexist in a single patient. Based on genetic and hormonal influences in congenital and acquired lymphological syndromes (e.g. lymphangiomatosis and AIDS), as well as the pathophysiological sequelae of lymphatic obstruction (oedema, ectasia, infection breakdown of tissue immunity, and neoplasia), proliferating endothelium or lymphangiogenesis emerges as a key process limiting these diverse phenomena and governing both the clinical manifestations and biological indolence or aggressiveness. Recent advances in in vivo and in vitro techniques make this process amenable to study.

Classification of Lymphoedema and Elephantiasis  
M. Földi

The classification of a disease has to serve practical purposes. It has to enable differential diagnosis and, as the second step, aetiological diagnosis. Diagnosis is not a final goal in itself but the conditio sine qua non of therapy. This means that:

(a) an established classification of a disease should be reconsidered and, if necessary, modified if there are changes in the principles of therapy;

(b) there should be a sound equilibrium between the invasiveness of the tools of diagnosis, on the one hand, and the risks and results of therapeutic methods, on the other.

Benign lymphoedemas of the limbs, if not complicated by a reflux of lymph or chyle, can be treated most successfully by decongestive physiotherapy, even in the elephantiasis stage. They should not necessarily be regarded as "surgical diseases".

If therapy is conservative, diagnostic methods have to be noninvasive (maximal restraint with lymphography). However, decongestive physiotherapy of lymphoedema requires the full facilities of a lymphology clinic with specialized medical lymphologists and physiotherapists.

It is proposed that Brunner's classification of lymphoedema into three stages, irrespective of its aetiology, should be generally accepted. Lymphoedema arises as a consequence of a low-output failure of the lymph vascular system, characterized by reduction of lymphatic transport capacity to a level which makes the reabsorption and transport of the normal lymphatic protein load impossible. As a consequence, protein-rich fluid starts to accumulate in the tissues. In the first stage, lymphoedema is pitting and is designated by Brunner as reversible; secondary tissue alterations are not yet present. The second stage has been called irreversible by Brunner; due to fibrosis and/or the deposition of fat, the oedema has lost its pitting character. A further increase in volume is by no means an obligatory component of this stage, which I myself call "spontaneously" irreversible because adequate conservative therapy abolishes first the oedema fluid and later the proliferated tissue as well. The third stage, lymphostatic elephantiasis, arises as a consequence of repeated inflammatory attacks, which cause tissue proliferation to reach monstrous dimensions.

Elephantiasis can be lobular or nonlobular, black or pale, but as a typical alteration dermal tissues become thick and as hard as cartilage. Even at this stage there still remains much protein-rich fluid in the depth of the tissues, perhaps encapsulated. This is by no means a pure pathological curiosity; quite the contrary, decongestive physiotherapy is based on the possibility that, in its first phase, the fluid can be evacuated, and this results in a rapid decrease of volume. This enables us to achieve a slow but continuous reduction of the size of the limb in the second phase of maintenance: continuous compression induces a remodelling of the limb: proliferated tissues become slowly stripped. If empty skin sacs remain, they may be removed by plastic surgery without hospitalization.

Aetiological classification of lymphoedema is congruent with the needs of therapy. Lymphoedema has to be classified into benign and malignant forms. A lymphoedema of the malignant type arises if the transport capacity of the lymph vascular system has been reduced by malignancy. Malignant lymphoedemas, of course, necessitate oncological treatment and the lymphoedema is only of secondary importance.

Every lymphoedema, in which transport capacity of the lymph vascular system has been reduced by any pathological alteration other than malignancy, belongs to the benign type. In these cases, aetiological diagnosis must determine whether filariasis is present or not. If the answer is yes, the first step in therapy should be eradication of the parasite. I do not see any reason why, after adequate parasitological therapy, these lymphoedemas should react in a different manner from the nonparasitic forms, but of course therapeutic trials are necessary.

Lymphoedemas of the limbs, complicated by reflux of lymph or chyle, and those of the genitalia, often necessitate surgical therapy.

Lymphatic Obstruction: Physiological and Therapeutic Aspects  
W.L. Olszewski

Normal pre- and postnodal lymph vessels in man are characterized by large, metabolically active, endothelial cells, subintimal collagen fibres, multiple muscular fibres and competent valves. As the vessel wall stretches with inflowing lymph, segments of vessel between two unidirectional valves contract spontaneously and rhythmically. This is the basic mechanism for propelling lymph into the collecting lymphatics. Normal lymph contains cellular elements, such as T-lymphocytes, a few monocytes and natural killer cells, and migrating Langerhans cells. Immunoglobulin concentration in lymph is evidently lower than in serum. Concentration of antibiotics or chemotherapeutics administered orally or intravenously is lower in lymph than in serum and its peak is always delayed.

Post-inflammatory obstructive changes in lymphatics and lymph nodes are characterized by obstruction of the lumen with thrombus, proliferation of fibroblasts, deposition of hyaline under the intima and destruction of muscle cells. Lymphatics lose their contractility. The flow is decreased and transudation of proteins through the wall occurs. The traffic of cells from the tissues to the regional lymph nodes is stopped and lymphangitis develops. The time of equilibration of antibiotic concentration between serum and lymph is significantly prolonged.

Observations on the pathophysiology of lymphatic obstruction, as well as clinical experience in conservative and surgical treatment, were presented. The results of trials of lymph vessel grafting and the reaction of lymphoid tissue to suture material were discussed.

CLINICAL AND PATHOLOGICAL ASPECTS OF FILARIAL INFECTION IN MAN

Tissue Changes in High-Protein Oedemas  
J.R. Casley-Smith

Oedemas have many deleterious effects on the tissues. They cause swelling, pain, loss of function (both at the gross and cellular levels), poor oxygenation of the tissues and delayed wound healing. When the tissues are swollen in oedema, initial lymphatics are dilated and do not collapse. High-protein oedemas show all the effects of low-protein oedemas, together with others additionally caused by the excessive amounts of protein in the tissues.

The effects of simple plasma protein excess have been demonstrated experimentally. Immunologically tolerant rats were given a pure high-protein oedema, via a subcutaneous injection of plasma from littermates (every 4 days for 64 days), care being taken not to cause the production of any mediators of inflammation. Polyvinylpyrrolidone (PVP), a non-metabolizable molecule of similar size, and saline were used as controls. When plasma was used, haemorrhage was frequent from about day 4 to day 32; it was not seen in the controls given PVP or saline. There were many open post-capillary venular junctions after plasma, a few after PVP, but none after saline. The blood capillaries contained many more vesicles and vacuoles in the groups treated with plasma and with PVP, although not in the group treated with saline. The number of blood capillaries was greatly increased in the plasma-treated group, less so in the PVP-treated one and unaltered in the saline group.

The macrophage population did not alter with saline; it was increased 20-fold with PVP and 40-fold with plasma. (The monocytes paralleled these to day 32, then declined again.) The use of a benzopyrone increased the numbers of macrophages even more -- to 40-fold with PVP and 150-fold with plasma -- and these macrophages were activated.

Neither saline nor PVP altered fibroblast numbers; protein increased them 125-fold; collagen fibres paralleled these alterations. With plasma, and to a lesser extent with PVP, there was a moderate lymphocytosis of small lymphocytes, but there were very few medium lymphocytes, T-lymphocytes, plasma cells or polymorphs.

These alterations (including those of blood vessels, tissues and initial lymphatics) show all the features of chronic inflammation. They strongly support the hypothesis that one mediator for chronic inflammation is the accumulation of plasma proteins, perhaps somewhat altered by their stagnation in the oedematous tissues. The simple accumulation of plasma proteins, without any other mediators or immunological reactions, provides an adequate explanation for almost all the tissue changes found in chronic lymphostatic disorders.

In chronic lymphostatic disorders there are considerable alterations in the collecting lymphatics, smooth muscular hyperplasia, fibromuscular alterations and excessive fibrosis.

An acute lymphostatic disorder subsides after a few weeks and the region appears clinically normal. In both humans and experimental animals, chronic lymphostatic disorder occurs some weeks to years after the acute phase, often as a result of a very minor injury. It has been found that the tissues are clinically normal during this latent period but far from normal in their fine structure. The collecting lymphatics are dilated and very tortuous, with much oedema in their walls and around them, and with much protein and fibrosis in these regions. It is likely that the excess fibrosis contracts and obstructs the new collateral vessels. Probably the macrophages also become exhausted. Thus both paths of excess-protein removal fail. The effects of secondary infection might be added to the effects of protein accumulation.

#### The Life-Cycle of Lymphatic Filarial Parasites

A.S. Dissanaiké

A brief outline of this subject was presented in order to remind the participants of the various stages of the parasites involved, as well as their location and duration. Four larval stages occur, with four months between them: two in the mosquito vector and two in the human host tissues. The third larval stage (L<sub>3</sub>), or infective stage, enters the tissue from the proboscis of the mosquito, and its subsequent migrations and moults may partially explain the early pathogenic features of infection.

#### Clinical Differences in the Lymphatic Manifestations of Bancroftian and Brugian Filariasis

F. Partono

The clinical course of lymphatic filariasis begins with an acute stage, followed 10 to 15 years later by the chronic stage. The acute stage is characterized by episodic occurrence of adenolymphangitis and the chronic stage by obstructive lesions in the lymphatics. During the chronic stage, episodic adenolymphangitis indicates active infection. The clinical manifestations of malayan filariasis and timorian filariasis are similar, but they differ from those of bancroftian filariasis.

In brugian filariasis, episodic lymphadenitis occurs most frequently in the inguinal region, followed by a characteristic retrograde lymphangitis, abscess, ulceration and cicatrization. The disease may evolve completely, but may also heal spontaneously at different stages of the clinical course. Elephantiasis is usually located at the lower extremities below the knee but occasionally affects the arm below the elbow. Genital lesions are not observed.

The clinical manifestations of bancroftian filariasis are more extensive. In most endemic areas, the lymphatics of the male genitals are most commonly affected, leading to funiculitis, epididymitis and orchitis. Hydrocoele is the most common chronic lesion. Elephantiasis affects the whole leg, arm, scrotum, vulva and breast, in order of decreasing frequency. Chyluria is observed in most endemic areas but its prevalence is low.

Clinical Aspects of Bancroftian Filariasis: An Appraisal of Some Issues  
V. Kumaraswami

Bancroftian filariasis can manifest itself clinically in several ways. Patients with the chronic form of the illness usually present with a grossly swollen extremity, the lower limbs being more frequently involved than the upper. The clinical course of the illness is usually punctuated by several episodes of "fever" with accompanying adenolymphangitis. The cause of these fevers is unknown.

Genital involvement is a frequent accompaniment of the disease. A large majority of affected males have hydrocoele, while others may have acute episodes of epididymo-orchitis. In some cases the penis may be grossly distorted. Female external genital disease is less well documented, although the female breast has been known to be a site of filarial disease. Other manifestations include chyluria and tropical pulmonary eosinophilia.

One of the most compelling issues in clinical filariasis is the aetiology of filarial "fevers". Studies to identify bacteria in cultures or, alternatively, to provide indirect evidence of bacterial involvement may help to establish the role of bacterial infection in this condition. Monitoring levels of eosinophils or their products, such as eosinophil cationic protein (ECP), may resolve the issue of the possible parasitological origin of these fevers.

Since we do not understand why some individuals have a silent onset of the disease or why it is quiescent for a long period, it may be advantageous to undertake clinical epidemiological studies. Such studies could throw light on the reasons for the low prevalence of female external genital disease.

Lymphographic studies to define the architecture of the lymphatics may be useful to delineate the existence of alternative pathways that could be used when planning reconstructive surgery, and also to explain the preferential involvement of certain sites in this disease.

Immunological approaches to the problem should clarify several of the clinical issues raised. They would be directed to understanding the factors that modify the clinical expression of the disease by such means as blocking antibodies or prenatal sensitization.

Pathology of Filarial Elephantiasis and Hydrocoele  
D.H. Connor

During World War II, 38 000 American troops were exposed to bancroftian filariasis. Of these, 12 000 were infected and studies on this cohort

revealed that: (a) the incubation period was at least three months, with peak manifestations at eight months; (b) the main clinicopathological features were lymphangitis, lymphadenopathy and inflammation of scrotal contents; (c) patients had complete regression of symptoms after being removed from the endemic area.

The most common pathological lesion is lymphangitis of the spermatic cord, characteristically beginning near the inguinal ring and moving down the cord. Lymphangitis of the epididymis, tunica, female breast, and major lymphatic channels of the thigh and inguino-femoral regions and axillae may also be involved. Of special importance is lymphangitis of the lymphatics of lymph node capsules and of the connective tissues around the lymph nodes. This is the first level of involvement.

The second level of involvement is in the lymph node proper. Adult filariae in the capsule and subcapsular sinusoids of the node provoke a pyogranulomatous response, which heals by hyalinized scar tissue, thus obstructing afferent lymphatics. Other changes in the nodes include dilation of sinusoids which contain many histiocytes and usually at least a few eosinophils; capsular fibrosis with the capsule traversed by dilated lymphatics; fibrosis and thickening of the trabeculae; follicular hyperplasia followed by follicular atrophy; and increased numbers of paracortical lymphocytes and plasma cells.

Lymphatics become dilated, greatly thickened, inflamed and occluded. Obstruction may also develop in the afferent lymphatics entering the nodes and in the cortex of the lymph nodes proper.

The predisposition of the parasites for lymphatic channels of the inguino-femoral regions, pelvis and abdomen causes elephantiasis of the lower limbs and genitalia.

#### Pathophysiology and Treatment of Filarial Chyluria

V.N.P. Tripathi

Chyluria is a chronic filarial legacy. If severe and persistent, it may lead not only to lipid but also lymphocyte depletion, with resultant debilitation and even mortality. In a study of more than 500 patients managed over a decade and a half, mortality was 1%.

Alterations have been detected in biochemical, haematological and immune system profiles, in addition to symptomatology and morbid anatomy. Seventy-five percent of patients are between 20 and 50 years of age, two-thirds being men. Seasonal preponderance is in April/May and August/September; 66.7% have chyluria, 26.7% haematochyluria and 6.6% predominantly haematuria. While a third of patients with chyluria of six months' or less duration may show spontaneous relief, in 10% of patients it lasts for ten years or more. There is a singular absence of lymphoedema in chylurics. Fatalities are mostly in short-duration chylurics.

The average body weight in chylurics is 50 kg with depleted fat deposits. Urinalysis, apart from being positive for chyle and lymphocytes, may show microfilariae. Peripheral blood may show lymphocytopenia, eosinophilia or microfilariae. Excretory urograms may show vascular impressions and ureteric deviation, which is displaced away from the spine in the upper part and towards it at the sacral promontory and results from gross lymphangiectasia along large vessels. Cystoscopy shows a "milky" ureteric reflux on the left side in 70% of patients, on the right side in 10% and on both sides in 20%. Retrograde pyelography demonstrates a pelvilymphatic

fistula in 43% of cases. Lymphangiography shows pelvilymphatic fistulae in 90% of cases and has the added advantage of depicting bilaterality.

Urinary losses over 24 hours are: triglycerides, 3607 mg; phospholipids, 341 mg; cholesterol, 155 mg; proteins, 10.5 g [albumin (48.6%) and globulins (51.4%)]. The serum profile is: triglycerides, 87 mg%; phospholipids, 127 mg%; cholesterol, 151 mg%; proteins, 5.42 mg% [albumin (49.8%) and globulins (50.2%)].

The immune system shows diminished responses, depicted by purified protein derivative, Candida albicans and 2,4-dinitrochlorobenzene reactivity tests, further supported by blastogenic and other tests for cell-mediated immunity. IgG and IgM levels are low. The haematological profile reveals lymphocytopenia. Many chylurics show erythrocytosis with raised haemoglobin, packed cell volume and RBC count.

A histopathological study of 30 operative specimens showed simultaneous evidence of lymphatic obstruction in the form of dilated, thick-walled lymphatics and also neolymphangiogenesis in the form of capillary spaces lined by immature endothelial cells. Lymph nodes, obtained from the renal hilum and groin of 22 of the same patients, showed a variety of changes from follicular hyperplasia to follicular atrophy with glandular fibrosis. While liver, spleen and kidneys studied in ten open biopsies were normal, biopsies of the small intestine demonstrated gross lacteal dilatations in the villi and submucosa, which were also lymphoedematous.

While microfilariae were demonstrated variably in blood and urine, serodiagnostic procedures with the enzyme-linked immunosorbent assay (ELISA), indirect haemagglutination (IHA) and counterimmunoelectrophoresis (CIEP) tests were of little use in diagnosis, owing to the low immune profile in chylurics.

Management of short-duration chylurics, not exceeding six months, is conservative. It includes restriction of fat in the diet and repeated courses of DEC. In patients with persistent or severe chyluria and progressive weight loss, a renal hilar lymphatic disconnection with nephropaxy is performed. The operative results in more than 100 patients, followed up to a maximum of 15 years, show satisfactory results with no "true" recurrences. Bilateral ureteric efflux or a subsequent contralateral efflux of chyle calls for the same operation on the opposite side. Operative mortality was nil but post-operative morbidity was 2% from renal loss due to vascular complications -- venous thrombosis in one case and arterial spasm in another.

#### Lymphatic Nodules in Human Filariasis S.K. Kar

A study on lymphatic filariasis carried out in two villages of Orissa (India) endemic for W. bancrofti revealed nodules in the extremities of 50 of 1926 subjects examined. Clinical manifestations, microfilaraemia and filarial abscesses were observed in 37.2, 15.8 and 11.8% of the subjects, respectively. The nodules in the extremities appearing along the lymphatic channels showed movement. These nodules were 1-3 cm in diameter, subcutaneous, freely mobile and found at the mid-arm, medial aspect of the thigh and below the knee joint region. In a few cases, the nodules appeared in the axillae or groin as a painful mass associated with lymphangitis of the extremity. The nodules descended distally to the mid-arm, epitrochlear region and thigh, where they persisted for a long time in untreated cases. Of 50 subjects presenting with nodules, 23 were found to be microfilaraemic. Such nodules were also observed in 6 of 163 asymptomatic microfilaria carriers during follow-up examinations.

Following DEC therapy, the nodules diminished in size and gradually disappeared. None of the subjects had received previous antifilarial therapy. Nodules removed surgically revealed lymph node structures with granuloma formation and the presence of an adult parasite on histopathological examination. Seven persons with clinical filarial disease developed multiple bead-like swellings in their arms following chemotherapy (DEC). These tiny nodules also moved along the line of the lymphatic and disappeared near the wrist in a two-week period. Similar nodules were also observed in a third village in Orissa which was endemic for Brugia malayi. These nodules result from granuloma formation in lymphatic filariasis. Further studies are needed to understand the host's immunological response to the occurrence, persistence and descent of these nodules.

#### Methods of Treatment for Filarial Elephantiasis Used in China

Zheng Huijun

Four different methods of therapy for filarial elephantiasis are applied in China: heat and bandage treatment; mulberry leaf extract injection and bandage treatment; audiofrequency electrotherapy (i.e. the application of 50 cycles alternating electric current) and bandage treatment; and surgery. The effective cure rates of these treatments ranged from 30.6 to 77.4%. In order to verify the mechanism of heat and bandage treatment, lymphangiography, the <sup>131</sup>I-HSA clearance test and radioactive isotope scanning of regional lymph nodes were carried out in a few patients before and after treatment. They demonstrated recovery of the damaged lymphatics and re-establishment of lymphatic drainage.

On the basis of our practice, it is suggested that combined therapy, including bandaging, the softening of the tissues of the affected legs, filaricidal treatment, and the control of bacterial and fungal secondary infection, is necessary in order to bring about more effective results. Most patients have received DEC therapy at some stage before remedial treatment for elephantiasis is begun.

#### Indications and Success of a Surgical Approach to Filarial Elephantiasis

S. Jamal

Inguinal nodo-venous shunt (NVS) has given 90% success in 300 cases of filarial oedema of grade II and above in the immediate post-operative period. For grade III and IV elephantiasis, excisional procedures are required to sustain the reduction. In over 15 years of follow-up, the NVS has maintained patency, as shown by clinical observation of rapid reduction in the size of recurrent swelling after simple elevation of the limb for a day. There remains a fold of loose skin for further excision. Charles' procedure (i.e. excision of lymphoedematous tissue, including deep fascia up to muscle and periosteum and covering of the raw area with thick split-skin graft taken from the involved, oedematous skin) should be avoided for filarial elephantiasis in India.

#### Relationship of Microfilaraemic Status to Lymphatic Disease in Papua New Guinea

J.W. Kazura and K. Forsyth

The objective of this analysis was first to determine whether amicrofilaraemia is associated with obstructive lymphatic disease and second to assess the utility of the Gib 13 monoclonal antibody-based immunoradiometric assay in predicting the development of disease.

In a total population of 472 individuals residing in East Sepik Province, an area where Wuchereria bancrofti infection is endemic, there was a microfilarial (mf) carrier rate of 55%; the age-specific prevalence of

microfilaraemia increased from 36% in subjects aged 10 years or less to over 70% in those older than 31 years. There were 45 subjects with elephantiasis of an extremity and/or hydrocoele. The mf carrier rate in these "diseased" individuals was 85% (38 of 45 mf+); a group of 45 age-matched (median age 40-42 years) subjects without lymphatic disease had a similar microfilarial carrier rate (87% or 39/45). It thus appears that microfilaraemic status does not correlate with the development of severe lymphatic disease in a population of W. bancrofti-infected subjects.

In preliminary studies with the Gib 13 assay, we determined that (i) immature and mature female or male worms of B. malayi release the Gib 13 epitope into excretory-secretory products in vitro; and (ii) there is a quantitative relationship between the amount of Gib 13 released and the number of parasites present in the culture medium. We then found that the Gib 13 antigen index (AI) in infected subjects is stable over a one-year period (mean AI of 4.0 in 30 subjects examined initially in 1984 and again in 1985).

When the age-specific AI in amicrofilaraemic, "nondiseased" subjects was examined, the level increased from 2.9 in subjects 10 years old or less to 4.3 in 11-20 year olds; the value rose progressively in the 20-30 and 31-40 year age-groups (5.8 and 7.0, respectively). In subjects aged 41 years or older, the mean AI was 5.5. These data indicate that the Gib 13 level increases with age, as might be expected if adult parasites accumulate with longer periods of exposure to infective larvae. We finally compared the Gib 13 AI in 25 subjects with severe lymphatic disease with 23 age-matched controls without disease. The mean  $\pm$  SE in the former "diseased" group was  $3.6 \pm 1.1$  vs.  $6.7 \pm 1.1$  in the "nondiseased" group. These results suggest that the Gib 13 level is low in diseased subjects, perhaps as a manifestation of relatively low adult worm burdens in subjects with severe lymphatic disease. Extensive cross-sectional and, more importantly, longitudinal studies are needed to verify this.

Host Immune Reactions During the Early Stages of Infection with Brugia malayi  
W.F. Piessens, L. Kurniawan and I. Koiman

The thesis that variation in immune responses to parasite antigens among exposed individuals determines the clinical outcome of infections with lymph-dwelling filariae is based on cross-sectional studies of native residents of endemic areas. This interpretation of available data implies that all individuals reach a given clinical status via the same antecedent pathway. Not enough is known about the long-term natural course of lymphatic filariasis to accept this premise without further study. Prospective studies on natives of endemic areas or on previously unexposed immigrants into such areas would allow evaluation of host-parasite interactions during the early phase of infection and the establishment of causal relationships between immune responses and clinical outcomes.

The early results of a study on transmigrant populations can be summarized as follows. Antibodies to microfilarial somatic antigens can be detected 6-12 months before it is possible to demonstrate sensitization to T-lymphocytes by the in vitro assay of antigen-induced lymphocyte proliferation. Microfilarial extracts suppress mitogen-induced lymphocyte proliferation in recent immigrants, but the prevalence of this nonspecific type of immune suppression decreases with increasing duration of exposure. By contrast, filarial antigen-specific immune suppression is quite common among indigenous residents of endemic areas but is difficult to demonstrate in recent immigrants. Whether or not it will be possible to correlate these observations with the ultimate outcome of filarial infection remains to be determined.

Immunoregulation in Man and its Relationship to Lymphatic Pathology in Filariasis

T.B. Nutman

The manner in which the human host responds immunologically to filarial parasites is clearly critical in determining the wide range of clinical pathology seen among those living in regions endemic for the lymphatic forms of filariasis. The immunoregulatory determinants of this response are myriad, although genetic, environmental, cell-mediated, humoral and parasite-derived factors have been variously implicated. While filarial and other parasite antigens have been shown to be major histocompatibility complex (MHC) restricted and evidence of prenatal sensitization in filariasis has been demonstrated, large-scale studies examining the interaction of environmental and genetic factors are needed.

The regulation of B- and T-cell responses has been studied extensively; the evidence suggests that patients with chronic lymphatic obstruction are immunologically competent with respect to in vitro B-, T-cell and T-cell-subset responsiveness to parasite antigen, whereas those with the asymptomatic infection show parasite-specific anergy in vitro. This lends further credence to the hypothesis that the host immune response must be implicated in the pathogenesis of the chronic lymphatic form of filariasis. Finally, the levels of circulating immune complexes were shown to be elevated and the levels of circulating parasite antigen negligible in patients with chronic lymphatic obstruction. This again suggests that the immune response, manifested by increased antigen-antibody complexes and thus the inability to clear parasite antigen effectively, is in part responsible for the chronic "obstruction" seen in lymphatic filariasis.

PATHOLOGY AND IMMUNOPATHOLOGY IN EXPERIMENTAL ANIMALS

Pathogenesis of Lymphatic Lesions in *Brugia pahangi*-Infected Jirds

T.R. Klei

The kinetic mechanisms of the granulomatous inflammatory response within the lymphatics of *B. pahangi*-infected jirds were measured over a 180-day period in animals receiving single inoculations of 50 *B. pahangi* L3 and compared to those of jirds receiving four or eight similar inoculations over the same time period. Antibody responses, lymphocyte transformation responses and preliminary granulomatous responses to such *B. pahangi* antigen extracts were measured. The results indicate that eight multiple infections do not produce a protective resistance to infection in jirds; in general, peak numbers of lymph thrombi and maximal renal lymph node sizes occur between 48 and 118 days post infection and decrease after that time. Modulation of the intralymphatic granulomatous response corresponds to the pulmonary granulomatous response and to trends in lymphocyte transformation responses seen, but not to circulating antibody titres. These findings indicate that a distinct sequence of responsive states occurs in jirds and the time intervals of these were determined. The modulatory response does not appear to be overcome by multiple infections. Thus single subcutaneous induced infections at appropriately selected times will be useful for studying details of the pathogenesis of these lesions.

Observations of Lymphatic Pathology in Nude Mice Parasitized by *Brugia* spp.

A.C. Vickery, K.H. Albertine and J.K. Nayar

Congenitally athymic and immunodeficient nude (nu/nu) mice, chronically parasitized by adult subperiodic *Brugia malayi*, develop grossly apparent and progressive lymphangiectasis of subcutaneous lymphatics. Persistent lymph-

oedema and skin changes consisting of ulcers, fissures or hyperpigmentation are observed in some mice examined more than 200 days after subcutaneous inoculation with B. malayi-infective larvae. Viable adult worms and not microfilariae appear to be responsible for the development of this elephantoid appearance. Comparable changes are not observed when mice are chronically parasitized by similar numbers of B. pahangi or B. patei.

Histological examination of parasitized lymphatics reveals dilatation and tortuosity in the absence of luminal or interstitial infiltrates, and physical blockage is not apparent. However, immunological reconstitution of nude mice harbouring adult B. malayi results in an episode of acute lymphangitis and lymphadenitis, sometimes with lymphatic obstruction. Reconstituted mice produce B. malayi-specific antibodies, exhibit eosinophilia and kill the majority of their adult worm burden. Levels of circulating microfilariae are not affected.

Lymph aspirated from dilated lymphatics of nude mice harbouring adult B. malayi contains soluble worm products, is free of bacterial sepsis and endotoxin, and has a total protein content more than double normal values. Thus, it appears that adult B. malayi produce soluble factors which can directly affect parasitized lymphatics in the absence of thymus-dependent hypersensitivity. Furthermore, the susceptibility of nude mice to selective adoptive immunological reconstitution suggests that mechanisms of adult worm killing could be characterized.

#### Pathogenesis of Limb Oedema in Brugia-Infected Dogs B. Hammerberg

All of the 18 dogs in this study sustained moderate to high microfilaraemia for 18 months or more after a single large infective dose of B. pahangi. Upon reinfection of five dogs with very low or no microfilaraemia (at 24 to 66 months after the original infection), three did not develop a sustained microfilaraemia and two of these three developed limb oedema. These three dogs had markedly increased antibody reactions with two bands of antigens (52 000 and 58 000 Mr on SDS-PAGE) from Brugia adult products and adult homogenate extracts. This antibody response was not detected in dogs with high microfilaraemia at the time of reinfection.

Neither the chronic nor the transient limb oedema in reinfected dogs was associated with occlusion of lymph ducts in the infected limb, as shown by xeroradiographic lymphangiography. This suggests that local pathophysiological mechanisms, in addition to lymph duct occlusion, play a role in the development of oedema in these reinfected dogs.

By contrast, one of five dogs given very large doses of infective larvae in the paw developed chronic limb oedema starting 12 months after infection, which was associated with lymph duct occlusion. This dog had the highest microfilaraemia of all the dogs under study. Other dogs given large distal limb infections showed transient episodes of limb oedema, which were associated with duct occlusion but appeared to be resolved by the appearance of "new" lymph ducts bypassing the nonfiltering popliteal node.

The possible aetiology of lymph duct occlusion suggested by the results from these five dogs may be complicated by findings of a high antibody titre in the dog with limb oedema against two antigens (50 000-55 000 Mr) from microfilarial products. Preliminary results at this time indicate that the only other dog with a response to these antigens is the microfilaraemic reinfected dog that developed chronic limb oedema.

These conclusions, drawn from in-depth longitudinal studies on experimental infections, are necessarily preliminary, because of the limited number of dogs being monitored. However, it is obvious that the pathogenesis of filarial oedema is the result of more than one factor. We have illustrated the unique usefulness of the dog model in investigating some of these factors. Although we have not reported on recently begun studies of monocyte/macrophage and lymphocyte function, the size and accessibility of infected and noninfected limb lymph ducts for cannulation can be exploited in studies on local production and action of monokines and lymphokines.

#### Experimental Lymphatic Dysfunction Caused by *Brugia malayi*

A. Ewert

Domestic cats and patas monkeys can be infected with *B. malayi* in such a way that the developing and mature filarial nematodes are localized in regional lymphatics of the hind legs. Reaction to the parasites results in visible local oedema in cats and lymph node enlargement, inflammation of lymph vessels and disruption of normal lymph flow in both cats and monkeys.

Lymph flow patterns can be examined by direct observation following injection of lymph-staining dye and reflection of the skin, by X-ray following injection of radio-opaque contrast media and by lymphoscintigraphy. Changes in the lymphatic valves and walls of the vessels can be observed by direct observation, light microscopy, scanning electron microscopy and transmission electron microscopy. Thrombi that form in parasitized vessels can be examined using the same techniques.

Within 24 hours after infective *Brugia* larvae were placed in a drop of saline over artificial puncture wounds on the hind foot of experimental animals, the larvae had migrated to the periphery of the first intervening lymph node, namely the popliteal. As the parasites matured and increased in size, the vessels dilated, valves of the infected lymph vessels became incompetent and the worms migrated back towards the site of infection. The filariae remained within the regional lymph vessels of the hind legs. Oedema developed within a month of infection and persisted up to three months after the last infection. The contralateral uninfected hind legs remained normal in appearance.

Collagen accumulated around infected lymph vessels in cats and persisted for variable periods of time. Thrombus formation and changes in the vessels hindered normal lymph flow. Preliminary ultrastructural studies suggest that vesicles in the cytoplasm of endothelial cells lining the lymph vessels may play a role in lymph transport. Although gross oedema is not evident in infected monkeys, lymph flow alteration is demonstrated by lymphoscintigraphy.

#### Lymphatic and Splenic Pathology of *Brugia malayi* Infections in the Leaf Monkey (*Presbytis* spp.)

Joon Wah Mak

The primates *Presbytis melalophos* and *P. cristata* are highly susceptible to experimental infection with subperiodic *B. malayi*. All animals infected with 200-300 infective larvae subcutaneously over the ventromedial aspect of the thigh and followed up for more than six weeks became patent for microfilaraemia. Geometric mean microfilarial counts rose rapidly during the first month of patency and then tended to level off by the fourth month. Peak counts achieved were in excess of 1600 mf/ml. Developing worms were mainly found in the sacral or para-aortic lymph nodes and vessels and in the thoracic duct (90%), while 10% were found in the inguinal or popliteal lymph nodes and vessels and in other places. Adult worms were evenly distributed in the

sacral, para-aortic lymph nodes and vessels and in the thoracic duct (45%), and in the inguinal lymph nodes and associated vessels (45%), with the remaining 10% being seen in other areas. Live worms induced less pathology and were mainly associated with dilatation of the lymphatic channels, while marked inflammatory response and thrombosis were seen around dead worms. In 90% of microfilaraemic animals, gross and microscopic granulomatous changes were seen in the spleen; the intensity of these reactions was related to the rate of decline of microfilaraemia, indicating the role of the spleen in the destruction of microfilariae.

Immunology of Bancroftian Filariasis in the Leaf Monkey (*Presbytis cristata*)  
J.R. Campbell

This three-year project studied cellular and humoral immune responses in *Presbytis cristata* monkeys experimentally infected with *Wuchereria bancrofti*. Animals were given subcutaneous injections in the left inguinal region of 250 third-stage infective larvae (L<sub>3</sub>) of *W. bancrofti*. The L<sub>3</sub> were injected either as a single dose of 250 parasites or as ten injections of 25 parasites each. Blood was taken for examination of sera and cells at various times before infection, during the prepatent period and after patency was achieved.

During the first six months of infection the animals showed no significant change either in total serum IgG or IgM concentrations, or in specific anti-*W. bancrofti* antibody titres. Specific antibody titres were measured by ELISA, using excretory-secretory (ES) antigens from cultured L<sub>3</sub>-ES antigens, and various other fractions from L<sub>3</sub> and adult worms are currently being tested. While all lymphocyte transformation assays (LTA) revealed normal concanavalin A and pokeweed mitogen responses, lymphocytes from only 25% of the infected animals were stimulated to proliferate in vitro in response to a variety of *W. bancrofti* antigens. Cellular responses were not enhanced by removal of suppressor T-lymphocytes with OKT8 monoclonal antibody plus complement.

If the immune response to *W. bancrofti* antigens is in fact minimal in these animals, there are several possible explanations. First, the filarial worms may be capable of molecular mimicry, as has been demonstrated with schistosomes. Alternatively, the parasite may cover its surface with actual host proteins, such as albumin, to avoid immune recognition. A third possibility is specific immune suppression, as suggested by our LTA data.

These findings suggest that the immune response to *W. bancrofti* may also be subverted by the parasite or its products. Therefore the production of any vaccine based on parasite antigens, whether naturally derived from an infected animal model such as *Presbytis* or produced by recombinant DNA technology, should be approached with caution and only with a thorough understanding of the host response to these antigens.

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