12th INTERNATIONAL WHO/TDR/FIL CONFERENCE ON LYMPHATIC PATHOLOGY AND IMMUNOPATHOLOGY IN FILARIASIS, THANJAVUR, INDIA, NOVEMBER 18-22, 1985*

This conference brought together basic and clinical scientists from around the world to Thanjavur Medical College and its Filariasis Clinical Research Unit in southern India. In addition to formal presentations by leading filariologists and lymphologists and question and answer periods, the group engaged in extensive discussions, formulated recommendations, and forecast future directions for basic and clinical research. They also toured villages in the endemic area, participated in clinics, and observed operative treatment. Ongoing international collaborative research endeavors were continued and new efforts initiated.

Drs. Brian Duke, Secretary, Scientific Working Group on Filariasis and A.S. Dissanaike, Secretary, Steering Committee of the Scientific Working Group represented World Health

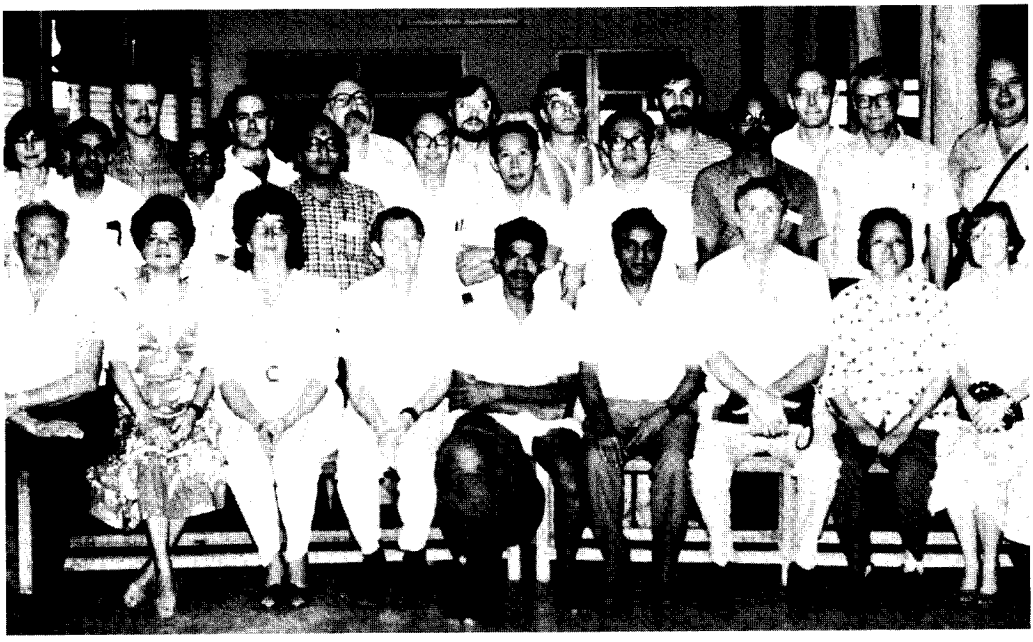

Fig. 1: Conference Participants: Front row (L to R): Michael Foldi, Hortensia Hornbeak, Marlys Witte, Eric Ottesen, patient, S. Jamal, Brian Duke, Zang Hui Jun, Ann Vickery. Middle row (L to R): A.S. Dissanaike, V. Kumaraswami, V.P. Tripathi, Adam Ewert, Felix Partono, J.W. Mak, S. Kar, Daniel Connor, John Casley-Smith. Back row (L to R): Mrs. Hammerberg, J.R. Campbell, G.J. Weil, Thomas Klei, J.W. Kazura, Willy Piessens, Thomas Nutman, Bruce Hammerberg (Photo by Nizar Jamal, courtesy of Dr. Daniel H. Connor).

*This report contains the collective views of an international group of experts convened by the UNDP/World Bank/WHO special program for research and training in tropical diseases (TDR). In the interest 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.

Fig. 2: Patients in Filariasis Clinical Research Unit at Thanjavur Medical College.

Fig. 4: Dr. S. Jamal (left), Professor of Plastic Surgery at Thanjavur Medical College and conference host, and Dr. Adam Ewert (second from left) pose with hospital medical and laboratory staff.

Fig. 3: Medical rounds in the nearby village of Vallam, Tamil Nadu, in the heart of the endemic region of filariasis.

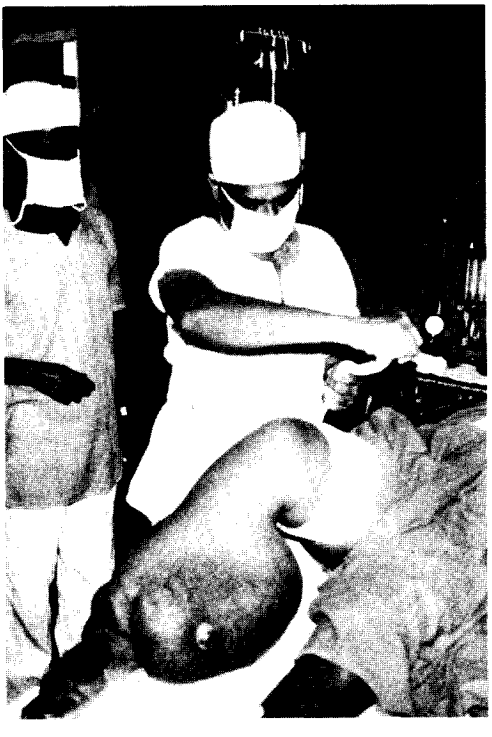

Fig. 5: Plastic surgeon G.V. Vivekanandan prepares a patient with filarial elephantiasis for a reduction operation at Thanjavur Medical College Hospital.

Organization Headquarters in Geneva, Switzerland, and Dr. Hortensia Hornbeak represented the co-sponsoring Fogarty International Center.

SUMMARY OF DISCUSSIONS

1.1 Pathogenesis of human lymphatic filariasis

1.1.1 Clinical aspects

The clinical manifestations of lymphatic filariasis, caused by adult or developing adult worms, are characterized during the acute stage by adenolymphangitis, usually accompanied by fever. Chronic obstructive lesions follow years later often after repeated attacks. These manifestations have been well described in brugian and bancroftian filariasis, but the underlying mechanisms which provoke 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 stage.Indeed the terms microfilaremia and amicrofilaremia need to be used with caution. A patient who is "amicrofilaremic" on a 20cu mm blood film may well be revealed as microfilaremic if 1-3ml of blood are examined by Nuclepore filtration. Furthermore, it can never be entirely excluded that "amicrofilaremics" (assessed on 1-3ml blood filtration) may harbor a reservoir of microfilariae in their lung capillaries, which are not released into the peripheral blood.

The differences in the anatomical distribution of the clinical manifestations of brugian and bancroftian filariasis may be due in part to the parasites having different tropisms 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.

The clinical differentiation between lymphedema and elephantiasis is often difficult to make and these signs are frequently grouped together. However, for clinical trial purposes it is necessary to adopt a uniform scheme of classification for lymphedema. While the following classification of lymphedema has been adopted by the International Society of Lymphology in Adelaide, 1985, its use in filariasis has not yet been tested.

Grade I: mostly pitting edema, some fibrosis, spontaneously reversible on elevation; *Grade II:* mostly non-pitting edema; much fibrosis; not spontaneously reversible on elevation; *elephantiasis:* much dermatosclerosis, in a Grade II lymphedema. The adjectives—mild, moderate, and severe can be applied to each category.

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

The three most compelling clinical issues that need to be investigated in lymphatic filarial disease would appear to be those concerning 1) the etiology of the acute episodes of adenolymphangitis with fever; 2) the risk factors that govern the acquisition of any form of the disease; and 3) the factors that govern the progression of the disease to its chronic form. Currently our knowledge regarding these problems is meager. These questions could be best answered by using a combination of clinical, epidemiological, microbiological, and parasitological techniques.

It is generally assumed that the microfilariae are not responsible for the obstructive lesions in the lymphatics in bancroftian or brugian filariasis. It is likewise assumed that the microfilariae, which are 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. However, there is no direct evidence for this and the possibility must be entertained that 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 readily be 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 lymphedema in filariasis are attributed to the presence of developing and adult worms, i.e., the L3 and L4 stages, which are relatively short-lived but which undergo a moult, and 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 lymphedema, but that some factor(s) produced by the living worms (and/or possibly the by-products of the L3 — L4 and La — young adult), coupled perhaps with interaction with the host's humoro-cellular inflammatory response, may be responsible for the malfunction of lymph drainage.

Recommendations

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

(a) 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 regarding environmental factors (e.g., seasonal variation in transmission; regions of the body bitten preferentially by the vector), other contributing factors (e.g., physical exertion, nutritional status), and sociological aspects (e.g., socioeconomic status, use of footwear and sleeping habits, etc. in relation to exposure to mosquito bites).

(b) To understand the pathogenesis of the adenolymphangitis of filariasis, studies are required to assess:
   (i) the role of possible bacterial infection;
   (ii) patterns of microfilaremia and the levels of parasite-derived products during and between acute episodes;
   (iii) the cells involved in parasite destruction or their constituent inflammatory products (e.g., eosinophils and eosinophil-derived products); and
   (iv) the host response to L3 antigens during such episodes.

(c) Approaches to examine 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.

1.1.2 Lymphological aspects

Although details of the causal mechanisms for the pathological conditions seen in filariasis are largely unknown, studies based on the interruption of lymphatic flow in other non-filarial human diseases, as well as those in animal models of lymphedemas, have indicated that the clinical spectrum of chronic filariasis can be explained in part by the high protein nature of the lymph accumulating in the tissues. Furthermore, the distribution of the lymphedema (e.g., distal extremity vs the entire extremity) might be explained by the presumed location of the lymphatic obstruction.

The lymphatics need not be physically obstructed in lymphedema; many factors can cause the collecting lymphatics either to go into spasm or to become widely dilated in flaccid paralysis. There are also great variations between individuals with regard to (i) the number of lymphatics and their anatomical arrangements; (ii) the number and activities of the proteolytic macrophages; and (iii) the resistance of the tissues to edema.

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

Recommendations

(a) Lymphoscintigraphic studies should be used to determine the level of obstruction in the various forms of lymphatic filariasis, such as limb edema, hydrocele, 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 and no genital disease) and bancroftian filariasis (disease often involving the entire limb; and genital disease common especially in males). Lymphoscintigraphic studies of individuals with asymptomatic infections should also be included.

(b) The use of simple non-invasive procedures (e.g., tonometric studies 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 lymphoscintigraphy to determine the expression of the disease developing in them.

(c) Studies of the constituents of lymphatic fluid are recommended as:

(i) they would help to resolve the issue of the existence of high protein edema in the "low output" failure of filarial lymphedema.
(ii) they would also 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.

(d) Training should be provided in clinical lymphological techniques so that adequate and early detection of lymphatic changes can be attained.
(e) Lymphologists should be encouraged to develop methods for investigating the lymphatic system which are suitable for field use.

1.1.3 Immunogenetic aspects

Differential susceptibilities to infection, and thereby differential immune responses among persons with filariasis, are manifested by differing clinical presentations. Thus, the genetic background of the endemic population becomes of interest, particularly in the light of the finding that filarial and other parasite antigens must be presented to the immune response in a fashion restricted by MHC products. Only two studies have addressed this issue directly among endemic populations. They used different experimental protocols and produced conflicting results. Thus there is a great need to define the genetic bases for the varied immune responses seen in endemic populations.

Recommendations

(a) family- and population-based studies should be carried out to:
(i) define the genetic background of patients with different clinical manifestations of filariasis (including chyluria), in particular with regard to:
- HLA-A, B, C typing;
- HLA-Dr and minor immune response loci;
- complement polymorphism
- other markers of genetic susceptibility; and
(ii) define the ability of each population to respond to externally administered vaccines, novel antigens, and recall antigens.

1.1.4 Modulation of the immune response

The wide range of clinical pathology seen among those living in regions endemic for the lymphatic forms of filariasis is felt 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. Thus, there is a need for comparative immunological studies across regional and parasitological boundaries.

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

A large body of work has 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 on patients with adenolymphangitis and elephantiasis, who characteristically respond to parasite antigens, need to be undertaken in order to examine how such a response may be modulated.

In view of the fact that both 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-idiotype antibodies or prostaglandins.

The questions that need to be addressed include:

(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 utilized for examining 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

(a) Methods to analyze qualitatively those antigens responsible for inducing both immune responses and immune tolerance to filarial parasites should be encouraged.
(b) Highly defined or clonal parasite antigens

should be used to allow a clearer picture of the host immune response to emerge.

1.1.5 Parasitological aspects

While the host response plays a role in determining the pathology of lymphatic filariasis, the parasite itself must be implicated as well, at least in part, in determining the varied pathology seen in human filarial infections. Although there are clearcut morphological distinctions among the three filarial parasites responsible for chronic lymphatic obstruction, the overlapping of clinical syndromes between areas of 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. Furthermore, 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 addressed 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 on the body may have on the site of inoculation of infective larvae.

The possibility that bouts of filarial fever and acute adenolymphangitis are time-related to the previous input of batches of infective larvae also needs to be borne in mind.

Recommendations

(a) The characteristics of the genetic and antigenic differences among isolates of the same species and between species needs to be undertaken. Once characterized, it needs to be determined whether these differences are responsible for the varied clinical conditions seen.

(b) Complete human post-mortem examinations are imperative in determining the functional and anatomical localization of adult parasites and microfilariae, as well as the pathology (in situ) associated with their deposition. Pathologists with the necessary specialized experience in the lymphatic system should be recruited to areas endemic for filariasis so that these issues can be examined.

1.1.6 Environmental aspects

Although it is felt that environmental factors are less likely than genetic factors to be responsible for determining how the host responds to the filarial parasite, nevertheless the former need to be examined, particularly in the light of work done both on in utero sensitization and on nutritional factors affecting the immune response.

Examination of environmental factors acting during the perinatal and neo-natal 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 environmental factors as they relate to the immune response to filarial parasites, such as nutritional, environmental, and sociological conditions, would be of great value.

1.2 Treatment and prevention of filarial lymphedema

1.2.1 Treatment of chronic lymphatic obstruction in filariasis

1.2.1.1 General

The treatment of lymphatic disease associated with Wuchereria bancrofti, Brugia malayi, or B. timori infection in humans represents a major therapeutic problem in endemic areas of Asia, Africa, and the Pacific region. There is currently not uniform approach to the management of the chronic disease manifestations of lymphatic filariasis, which include hydrocele, elephantiasis of an extremity, lymphatic inflammation/obstruction of the scrotum and its contents (epididymitis, funiculitis, testicular involvement) and chyluria.

It is highly encouraging that there now appear to be several treatment methods, both 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 are, however, unclear. The various methods of treatment that may be useful for managing each of the disease manifestations are summarized below, and recommendations for investigations aimed at defining the optimal method(s) of treatment are presented.

The questions whether it is the live or the dead adult worms that cause more damage to the functioning of the host's lymphatic drainage is

vital when filaricidal therapy with diethylcarbamazine citrate (DEC) or other drugs is considered. In general, from the results of work on animal models (including the nude mouse) and from experience with DEC chemotherapy in man, it appears that macrofilaricide treatment has a beneficial rather than a detrimental effect on acute adenolymphangitis and lymphedema. This is certainly true in the earlier stages of infection; but in the later stages, when gross obstructive damage has led to fibrotic lymphedema and elephantiasis, macrofilaricidal treatment may have very little or no beneficial effect. Treatment then depends entirely on reestablishing lymphatic drainage by surgical and physiotherapeutic means.

The important conclusion (or at least the best justified 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 lymphedema than a laissez-faire "non-filaricidal" policy based on the belief that dead or dying adult worms are the cause of the lymphatic malfunction.

1.2.1.2 Hydrocele

(a) Definition and pathophysiology

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

(b) Treatment

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

(c) Recommendations

(i) The optimal surgical procedure for management of filarial hydrocele needs to be defined.

(ii) The possible utility of DEC therapy in the management of filarial hydrocele needs to be examined, particularly in areas where surgery is unavailable.

1.2.1.3 Scrotal lymphedema and lymphatic inflammation/obstruction of its contents (epididymis, spermatic cord, testes)

(a) Definitions and pathophysiology

These conditions include lymph collection in the testis, epididymitis, funiculitis (swelling of spermatic cord), and thickening of the scrotal skin. No homologous lesions have been reported in females involving the ovary or fallopian tubes.

(b) Treatment

During the acute phase of a filarial "attack" on these organs, a conventional course of DEC should be given and followed by periodic administration thereafter to prevent recurrent attacks. This approach may be helpful in subjects without longstanding 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 regimes in reduction of genital lymphedema should be investigated.

(ii) Standard surgical procedures for drainage should be established.

1.2.1.4 Elephantiasis of the extremities

(a) Definition and pathophysiology

Recurrent episodes of limb lymphedema, resulting first in pitting edema and then in non-pitting edema 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 is swollen. Secondary infections of the skin (bacterial and fungal) are common in these individuals, particularly 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 smelly lymphorrhea.

(b) Treatment

Studies in areas of Indonesia where Brugia timori infections exist indicate that DEC ad-

ministered over a long period of time (more than 1 year) results 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 lymphedema by stimulation of macrophages to remove protein and produce collagenase that will dissolve fibrosis.

Similar observations using "high" doses of DEC (3.0-4.2g daily for 7 days in W. bancrofti, or 1.5-2.0g daily for 7 days in B. malayi) have been made in Chinese subjects.They suggest that this drug, especially when used in conjunction with compressive dressings, may be beneficial. Bandaging of the extremity with heat treatment and injection of mulberry leaf extract have also been reported to be beneficial in Chinese subjects.

Striking beneficial response with regression of elephantiasis have also been observed with lymphnodovenous and 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 done under local anesthesia, 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 anti-fungal 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 lymphedema, with or without elephantiasis (bancroftian and brugian), needs to be studied in a controlled fashion. The optimal dose of drug needs to be established.

(ii) The utility of surgical approaches such as lymphnodo- or lymphovenous drainage in large populations needs to be established. This includes studies on the feasibility of this surgery in regional centers where resources are limited.

(iii) The possible application of physiotherapy as an adjunct in management should be explored.

(iv) The possible efficacy of benzopyrones in regression of filarial lymphedema in animals and in man should be examined.

(v) The possible role of steroids and anti-inflammatory agents in reversing disease should be examined.

(vi) The mechanism of action of DEC in reversing lymphedema in human and experimental animal models needs to be defined.

(vii) The efficacy 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 effects of such treatment, repeated with each attack, on the development of chronic lymphedema, and on the frequency with which acute episodes recur.

1.2.1.5 Chyluria

(a) Definition and pathophysiology

Chyluria may be defined as the excretion of chyle, along with lymph, in the urinary tract. A minority of affected subjects may also have hematuria. 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 location 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 possible fat-soluble vitamins are excreted and lead to weight loss.

(b) Treatment

The approach to treatment is currently based on the clinical course in the individual patient. In subjects with short-term (less than 6 months) low-grade chyluria, conservative approaches utilizing DEC therapy and restriction of dietary fats may be helpful. In the majority of subjects whose chyluria continues despite these measures, surgical approaches are indicated. These include disconnection of the renal hilar lymphatics with nephropexy. Surgical procedures such as nephrectomy, renal capsular decortication, and the periureteric disconnection ("Patna operation") should not be performed.

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. In addition, studies in China suggest that a mixture of herbal medicines plus DEC may be beneficial.

(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 in a quantitative manner.
(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 centers are available.
(v) The prevalence of chyluria in various endemic areas needs to be determined.
(vi) Animal models of chyluria need to be developed.
(vii) The active pharmacological principle(s) in mulberry leaf extract, used in treatment of chyluria, should be investigated and their mode of action defined.

1.3 Animal models of filariasis and their relevance to the pathogenesis and treatment of human disease

1.3.1 General

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

1.3.2 Models for acute and chronic filarial disease

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

1.3.2.1 Acute filarial disease

In man this condition is considered to be 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 upon research using animal models of acute filarial disease.

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 lymphedema occur in Brugia — cat or dog model systems.Nude mice reconstituted with splenic lymphocytes from heterozygous litter-mates also show an acute lymphangitis.These symptoms should be utilized to study this disease condition. It would also be useful to study acute reactions in animals with different immune states; i.e., (i) those resistant via use of irradiated infective larvae; (ii) those immune, but not resistant, via passive sensitization with antigen; and (iii) non-immune 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

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

1.3.2.2 Chronic obstructive lymphatic disease

Lymphedema and resulting elephantiasis are considered to be the results of obstructive inflammatory reactions induced by filarial infections. The sites of these obstructive lesions, which may occur with or without accompanying lymphedema, should be studied and compared with obstructions that occur in non-filarial lymphedema. It is noteworthy that in dogs with artificial ligature of the lymphatics there is first a short-lived edema which then clears and is followed only after a year or more by chronic edema. During the latent phase there may be striking lymphographic changes despite the lack of edema, and these findings indicate that a very long follow-up is often needed to detect the development of chronic clinical lymphedema.

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 lymphedema which have shared features with non-filarial lymphedema. 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 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 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 lymphedema has been less well studied. However, several models are suitable for this purpose. They include 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 lymphedema 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 lymphedema. Contemporary lymphological methods should be used, but attempts should be made to develop new quantitative methods. Further basic information on the workings of the normal and obstructed (filarial and non-filarial) 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 lymphedema, as well as the participation of endothelial cells and lymphocytes in the process.

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

An important question regarding the development of lesions in B. malayi and W. bancrofti in man is the role which the site of vector feeding may have on subsequent lesion development. Studies on the site of infection and on possible lymph tropisms of filariae should be conducted. Several models would be useful for this including Brugia spp. in dogs, cats, monkeys, and jirds.

Recommendation

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

1.3.3 Recently discovered and specialized models

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

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

The pre-patent interval in this infection is between 5 and 11 months, most commonly 7-8 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 insight into the pathogenesis of human disease. The potential of the W. kalimantani/presbytis spp. model has not been fully utilized and it is recommended that workers in endemic areas should be encouraged to explore this together with experimental and chemotherapeutic studies. The potential for studying the induction of acute filarial disease in the W. kalimantani/Presbytis spp. model should be explored.

Recommendation

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

1.3.3.2 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 characterization 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 man, with dilatation and tortuosity of parasitized lymphatics, lymphedema 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 lymphedema 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 upon the development of filarial lesions. Nude mice harboring 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 (E-S) antigens produced by worms living in a physiologic environment. The analysis and characterization of these products, uncomplexed by products of the host's immune response, is simplified compared to conventional hosts. Examination of the direct effects of purified worm products upon lymphatics of animals in vitro and upon lymphatic explants on cells in vitro is recommended.The effects of DEC and of macrofilaricidal arsenicals on the adult worms of B. malayi in nude mice need investigation, and the effects of hemolytic streptococcal infection in producing lymphedema in these animals could be studied. Comparison with normal mice should also be made, as well as exploring the possibility of infecting with B. malayi other strains of mice having different cellular immunological deficiencies.

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

The other important species of filariids which develop in man or animals should be screened systematically for 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 pathogenicity in the host, and investigations into its susceptibility to chemotherapeutic agents.

Recommendation

(i) The nude mouse model should be used for studying the pathogenesis of filarial lymphatic obstruction; for its potential in chemotherapeutic screening; and for its ability to support development of O. volvulus and other human filarial parasites.

ABSTRACTS OF PRESENTATIONS

1. Physiology and pathophysiology of the lymph circulation in man with a note on lymphangiogenesis and lymphologic syndromes

MH Witte, and CL Witte, Department of Surgery, University of Arizona, Tucson, Arizona

Two conceptual frameworks are introduced to put filariasis in the perspective of the broad discipline of lymphology, the study of the integral workings of lymphatics, lymph nodes, lymph, and lymphocytes in health and disease.

a. A unifying concept termed the "lymph imbalance theory" views all interstitial edema or serous effusion as ultimately a disturbance in circulation of extracellular fluid where net capillary filtration or 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 bedside 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 edematous 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 lymph absorption and evaluated in terms of restoring the balance.

b. Lymphedema, lymphangiectasia, lymphatic tumor 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 lymphologic syndromes (e.g., lymphangiomatosis and AIDS) as well as the pathophysiologic sequelae of lymphatic obstruction (edema, ectasia, infection, breakdown of tissue immunity, and neoplasia), proliferating endothelium or lymphangiogenesis emerges as a key process linking these diverse phenomena and governing both the clinical manifestations and biologic indolence or aggressiveness. Recent advances in techniques both in vivo and in vitro make endothelium and lymphangiogenesis more amenable to study.

2. Classification of lymphedema and elephantiasis

M Foldi, International College Clinic of Lymphology, Freiburg, FRG

Classification of a disease should facilitate both differential diagnosis and etiological diagnosis. Although diagnosis, per se, is not a final goal, it is a sina qua non for effective therapy. This approach means that:

1) an accepted disease classification is subject to change if principles of therapy improve and vary
2) there must be a sound balance between the invasiveness or tools of diagnosis, on the one hand, and risks and benefits of therapeutic methods on the other.

Based on personal experience during the past 8 years in management of lymphedema, I have acquired considerable evidence that benign (see later) lymphedemas of the limbs, if not complicated by a reflux of lymph or chyle, can be treated, even in the elephantine stage, by decongestive physiotherapy with striking success. This type of lymphedema is not a "surgical disease".

If therapy is conservative (i.e., non-operative), diagnostic methods are preferably non-invasive (i.e., maximal restraint with lymphography). Despite the title designated by World Health Organization it is essential to recognize the following:

1) Decongestive physiotherapy of lymphedema is not new: it is based on principles described by Winiwarter in 1892.
2) The average duration of the first decongestive phase of treatment, which is preferably performed in a facility comparable to my Clinic of Lymphology, is 4 weeks and must be followed by a second phase of "maintenance and optimalization".
3) Decongestive physiotherapy is based on four requirements:
   a. Experienced lymphologists, that is, physicians preferably with specialization in internal medicine and oncology and additional subspecialization in lymphatic disorders.
   b. Physiotherapists who have undergone a 4-week course followed by at least 2-week practice in a Clinic of Lymphology.
   c. Availability of hygienic measures, antifungal drugs, ointments, bandaging materials, and elastic stockings/sleeves.
   d. Full compliance of the patient who has been taught and educated during the first (decongestive) phase.

The usual classification of lymphedemas into primary and secondary forms, and the classification of primary lymphedema into hypoplastic, hyperplastic, etc. groups has little or no practical consequence if therapy is identical, i.e., conservative. Surgeons who favor creation of lymphovenous shunts usually request lymphography to differentiate between hypo- and hyperplastic forms, but as Clodius has shown, the beneficial effects of these operations are only transitory. Moreover, the number of specialists in this branch of surgery plus the duration of these interventions on the one hand and the huge number of patients on the other illustrates that lymphatic microsurgery is not suited for the solution of lymphedema. It must also be recognized that patients in the second and third stage—according to Brunners classification (see later)—have to be excluded as potential candidates for lymphatic microsurgery.

Brunner's classification of lymphedema into 3 stages—irrespective of etiology—should be generally accepted. In order to understand this classification, lymphedema must be defined:

Lymphedema arises as the consequence of a low-output-failure of the lymph vascular system characterized by a lymphatic transport capacity reduced to a level which makes reabsorption and transport of the normal lymphatic protein load impossible, As a consequence, protein-rich fluid starts to accumulate in the tissues. In this first stage lymphedema 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 edema has lost its pitting character. A further increase in volume is by no means a prerequisite of this stage I call "spontaneously" irreversible because adequate conservative therapy abolishes first the edema fluid and, later, the proliferated tissue, too. The third stage, lymphostatic elephantiasis, arises as a consequence of repeated inflammatory attacks causing tissue proliferation to reach monstrous dimensions.

Elephantiasis can be lobular or non-lobular, dark or pale, but typically dermal tissues become thick and hard as cartilage. Even in this stage, there still remains much protein-rich fluid in the depth of the tissues, perhaps encapsulated—a fact known to pathologists of the 19th century. This complication is not simply a pathological curiosity because decongestive physiotherapy is based on the possibility that, in the first phase, the fluid can be evacuated with rapid decrease of volume. This response facilitates a slow, but continuous reduction of the size of the limb during the second phase of "maintenance and optimalization": continuous compression induces remodeling of the limb: proliferated tissues become slowly stripped. If empty skin sacs remain, they may be removed by simple excision in an outpatient setting without hospitalization.

Returning to etiological classification which follows is integral to effective therapy. Lymphedema is preferably classified into benign and malignant forms. Lymphedema of the malignant type arises when the transport capacity of the lymph vascular system has been obliterated by carcinoma or other malignancy.The principal value of etiological diagnosis rests on this distinction in that: malignant lymphedema necessitates oncological treatment; lymphedema is only of a secondary importance. All other lymphedemas belong to the benign type. In these latter instances further etiologic "breakdown" relates to whether filariasis is present.If it is, then the first step in therapy aims to eradicate the parasite. Once adequate parasitocidal drugs are administered, these lymphedemas should respond similarly to non-parasitic forms (see above), but therapeutic trials are nonetheless necessary. The only other issue in etiologic diagnosis is whether lymphedema is factitious: some patients actually "strangulate" their own limbs.

Finally, lymphedema complicated by reflux of lymph or chyle and that involving the genitalia, often requires surgical therapy.

ETIOLOGIC CLASSIFICATION OF LYMPHEDEMAS

1. Benign
   - *1.1 Filariasis* +
   - 1.2 Filariasis —
   - 1.3 Artificial
2. Malignant

3. Tissue changes in high-protein oedemas

JR Casley-Smith, Henry Thomas Laboratory, University of Adelaide, Australia

Oedemas have many deleterious effects on tissues including pain, loss of function (both at the gross and cellular levels), poor oxygenation, and delayed wound healing. Moreover, when tissues are swollen initial lymphatics dilate and do not collapse.

High-protein oedemas produce all the effects of low-protein ones, plus others specifically attributable to excessive amounts of protein in the tissues.

The effects of simple plasma protein excess have been demonstrated experimentally. Taking care not to stimulate mediators of inflammation, immunologically-tolerant rats were given a pure high-protein oedema via subcutaneous injection of plasma from littermates (every four days for 64 days). PVP, a non-metabolized molecule of similar size, and saline served as controls. Then plasma was used, hemorrhage was frequent—from about the 4th until the 32nd day, but was not seen in the 4 controls given PVP or saline. There were many open post-capillary venular junctions after plasma, a few after PVP, none after saline. The numbers of blood capillaries were 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 change with saline but was increased 20 times with PVP and 40 times with plasma. Monocyte changes paralleled macrophage changes up to 32 days and then declined. Benzo-pyrone increased these numbers even more—to 40 times with PVP and 150 times with plasma. These macrophages were activated. Neither saline nor PVP altered fibroblast numbers: protein increased them 125 times! Collagen fibers paralleled these alterations. With plasma and to a lesser extent with PVP, there was a moderate lymphocytosis of small

lymphocytes, but few medium lymphocytes, T-lymphocytes, plasma cells, or polymorphs.

These alterations (including those of the blood vessels, above, and of the initial lymphatics, below) encompass the main features of chronic inflammation. They strongly support Willoughby and Di Rosa's (1970) hypothesis: that one mediator for chronic inflammation is the accumulation of plasma proteins, perhaps altered by stagnation in the oedematous tissues. Thus, mere accumulation of plasma proteins, without other mediators or immunological reactions, promotes tissue changes characteristic of chronic lymphostasis.

In chronic lymphostasis there are considerable alterations in collecting lymphatics, smooth muscle hyperplasia, fibromuscular alterations, and excessive fibrosis.

The alterations in the tissues produced by simple injections of protein (above) are almost identical with those of subacute and chronic lymphoedema. There is an increase in the levels of macrophage enzymes. Often pale areas are seen around these cells, perhaps indicating extracellular proteolysis. However, since the numbers of macrophages increase far more than their enzymes do, many of these cells must be unstimulated. They have fewer pseudopodia than normal, and considerably more vacuoles, i.e., they are relatively loaded and inactive.

An acute lymphostatic disorder subsides after a few weeks and the region appears clinically normal. In both humans and experimental animals, overtly chronic lymphostasis occurs weeks to years after the acute phase, often precipitated by minor injury. Although the tissue appears grossly normal during the latent period, it is far from normal in its ultrastructure. Collecting lymphatics are dilated and tortuous with much oedema protein and fibrosis within their walls and around them. Most likely excess fibrosis favors contraction and obstruction of new collateral channels. Probably, too, macrophages become exhausted. Thus both paths of excess-protein removal fail. To these effects of protein accumulation are added secondary infections, lymphangitis, and lymphatic obliteration.

REFERENCES:

Casley-Smith,J.R., Casley-Smith, Judith R.: "High-Protein Oedemas and the Benzo-Pyrones", Lippincott, Sydney and Balt., in press (1985).

Willoughby,D.A., Di Rosa, M.: A unifying concept for inflammation. In "Immunopathology in Inflammation", Excerpta Med. Int. Cong. Series No. 229,28-38.

4. Lymphatic obstruction—Physiological and therapeutical aspects

WL Olszewski, Dept. of Surgical Research and Transplantology, Medical Research Center, Polish Academy of Sciences, Warsaw, Poland

Normal pre- and post-nodal lymph vessels in man are characterized by large, metabolically active endothelial cells, subintimal collagen fibers, multiple muscular fibers, and competent valves. Segments of vessel between two unidirectional valves contract spontaneously and rhythmically upon stretching the wall with inflowing lymph. This is the basic mechanism for propelling lymph in lymphatics. Normal lymph contains cellular elements such as T-lymphocytes, few monocytes and NK cells, and migrating Langerhans cells. Immunoglobulin concentration in lymph is lower than serum. Concentrations of antibiotic or chemotherapeutic drugs administered orally or intravenously is lower in lymph than in serum and peaks delayed.

Postinflammatory 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. Flow is decreased and movement of proteins through the wall occurs. The traffic of cells from the tissues to the regional lymph nodes is impeded 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 with non-operative and operative treatment were described. The results of trials of lymph vascular grafting and the reaction of lymphoid tissue to suture material were discussed.

5. Clinical differences in the lymphatic manifestations of Bancroftian and Brugian filariasis

F Partono, Department of Parasitology, University of Indonesia, Jakarta, Indonesia

The clinical course of lymphatic filariasis can be divided into the acute stage, followed by the chronic stage 10 to 15 years later. 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 lym-

phadenitis occurs most frequently at the inguinal region, followed by a characteristic retrograde lymphangitis, abscess formation, ulceration, and cicatrization. The disease may evolve completely but may also heal spontaneously at different stages of the clinical course. Elephantiasis is characteristically located below the knee, but occasionally affects the arm below the elbow. Genital lesions are not observed. The clinical manifestations of Bancroftian filariasis in contrast are more extensive. In most endemic areas, the lymphatics of the male genitalia are most commonly affected, leading to funiculitis, epididymitis, and orchitis. Hydrocele is the commonest chronic lesion. Elephantiasis affects the entire leg, arm, scrotum, vulva, and breast, in order of decreasing frequencies. Chyluria is observed in most endemic areas but the prevalence is low.

6. Clinical aspects of bancroftian filariasis: An appraisal of some issues

V Kumaraswami, Tuberculosis Research Centre, Madras, India

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

Genital involvement is a frequent accompaniment of the disease. A large majority of males have a hydrocele while others many have acute episodes of epididymorchitis. Sometimes the penis is grossly distorted. Female external genital involvement is rarer although the female breast is known to be the seat of filarial disease. Other manifestations are chyluria and tropical pulmonary eosinophilia.

A compelling issue in clinical filariasis is the etiology of filarial "fevers". Studies to identify bacteria in cultures or alternatively identification of indirect evidence of bacterial involvement may help establish the role of bacterial infection in this situation. Monitoring levels of eosinophils or the byproducts such as eosinophil cationic protein (ECP) may resolve the issue of possible parasitologic origin of these fevers.

Because it is unclear why some individuals have a silent onset of the disease or why it is quiescent for long periods, it may be advantageous to conduct clinical epidemiologic studies to clarify these issues. Such studies may also explain the rarity of female external genital involvement.

Lymphographic studies to define lymphatic architecture may delineate alternative pathways to help plan profitably reconstructive surgery and also to evaluate preferential involvement of certain sites in this disease.

Immunological approaches should be directed at understanding the factors that modify clinical expression of filariasis including blocking antibodies and prenatal sensitization.

7. Pathology of filaria elephantiasis and hydrocele

DG Connor, Dept. of Infectious and Parasitic Diseases, AFIP, Washington, DC

During World War II 38,000 American troops were exposed to filariasis. Of these, 12,000 were infected and studies on this cohort revealed that 1) the incubation period was at least 3 months with peak manifestations at 8 months; 2) the main clinico-pathological features were lymphangitis, lymphadenopathy, and inflammation of scrotal contents; and 3) patients had a 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, of the tunica, of the female breast, and major lymphatic channels of the thigh, inguinal-femoral region, and axillae may also be involved. Of special importance is lymphangitis in the capsule of lymph nodes and in 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 filariasis in the capsule and subcapsular sinusoids of the node provokes a pyogranulomatous response which leads by hyalinized scar tissue—thus obstructing afferent lymphatics. Other changes in the nodes include 1) dilated sinusoids containing many histiocytes and usually at least a few eosinophils; 2) capsular fibrosis with the capsule transversed by dilated lymphatics; 3) fibrosis and thickening of the trabeculae; 4) follicular hyperplasia followed by follicular atrophy; and 5) 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 for lymphatic channels of the inguino-femoral regions, the pelvis, and abdomen causes elephantiasis of limbs and genitalia.

8. Pathophysiology and treatment of filarial chyluria

TNP Tripathi, Division of Urology and Department of Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Chyluria is a chronic filarial legacy. It may lead, if severe and persistent, not only to lipid but also lymphocyte depletion, with resultant debilitation and even mortality observed in 10% of more than 500 patients managed over a decade and a half.

Phase-wise studies have depicted alterations in biochemical, hematological, and immune system profiles in addition to symptomatology and morbid anatomy. Seventy-five percent are between 20-50 years, ⅔ are men. Seasonal preponderance is in April/ May and August/ September; 66.7% have chyluria, 26.7% hematochyluria, and 6.6% predominantly hematuria. While a third of patients with chyluria of 6 months or less duration may show spontaneous regression, in 10% it lasts 10 years or more. There is a singular absence of lymphedema in chyluria. Fatalities are mostly in short-duration chylurias.

The average person weighs 50kg with depleted fat depots. 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 deviations, which is away from the spine in upper part and towards the sacral promontory, resulting from gross lymphangiectasia along large vessels. Cystoscopy shows "milky" ureteric reflux on the left side in 70%, right side in 10% and both sides in 20%. Retrograde pyelography demonstrates pelvi-lymphatic communications in 43%—the demonstration rate of this "sign of chyluria" becomes 90% on lymphangiography with added advantage of depiction of bilaterality, lymphangiomatosis in renal hilar area and retroperitoneum in the majority.

Average 24-hour urinary losses were: triglycerides 3607mg; phospholipids 341mg; cholesterol 155mg; protein loss was also considerable. Serum profile was: triglycerides 87mg%; phospholipids 127mg%; cholesterol 151mg%; proteins 5.42gm%—49.8% albumin and 50.2% globulins.

The immune system showed diminished responsiveness, depicted by P.P.D., candida albicans, and 2:4 DNCB reactivity tests and further supported by blastogenic and other tests for cell-mediated immunity. IgG and IgM levels were low. Hematological profile revealed lymphocytopenia. Many chyluric patients showed erythrocytosis with raised hemoglobin and packed red cell volume and erythrocyte count.

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 in these patients varied from follicular hyperplasia to follicular atrophy with fibrosis. Liver, spleen, and kidneys in 10 open biopsies were, however, normal. In the small intestine there were grossly dilated lymphatics in villi and submucosa, which were also edematous.

While microfilariae were demonstrated variably in blood/urine, serodiagnostic procedures like ELISA were inconclusive probably due to low immune profile in chyluric patients.

Management of short-duration chylurics, not exceeding 6 months, was conservative and consisted primarily of dietetic fat restriction and repeated courses of DEC. In patients with persistent or severe chyluria with progressive weight loss, renal hilar lymphatic disconnection with nephropexy was performed. The operative results in more than 100 patients followed up to a maximum of 15 years showed satisfactory result with no "true" recurrences. Bilateral ureteric efflux or a subsequent contralateral efflux of chyle necessitated the same operation on opposite side. The operative mortality was 0% and morbidity was 2% in the form of renal loss due to vascular complication—venous thrombosis in one and arterial spasm in another patient.

9. Lymphatic nodules in human filariasis

SK Kar, Regional Medical Research Center, Bhubaneswarr, India

A study of lymphatic filariasis carried out in two villages of Orissa (endemic for W. bancrofti) revealed nodules in extremities of 50 subjects out of 1926 examined. The clinical manifestations, microfilaremia, and filarial abscesses, were observed in 37.2, 15.8, and 11.8 percent of subjects respectively. Nodules in the extremities appeared along lymphatic channels and seemed to migrate. The nodules were 1-3cm in diameter, subcutaneous, freely mobile, and found at the midarm, medial aspect of the thigh, and below the knee. In a few, the nodules appeared in the axillae or groin as a painful mass associated with peripheral lymphatics. The nodules descended distally to mid-arm, epitrochlear region and thigh where they persisted for a long time in untreated patients. Following diethylcarbamazine (DEC) therapy, nodules diminished and gradually disappeared. None of these subjects received

antifilarial therapy before. Nodules removed surgically revealed lymph node structure with granuloma formation and presence of adult parasite on histopathologic examination. Seven persons with clinical filariasis developed multiple bead-like swellings in the arms following chemotherapy (DEC). These tiny nodules also "moved" along the lymphatic line and disappeared near the wrist in a two-week period. Similar nodules resulted from granuloma formation in lymphatic filariasis. Further studies are needed to understand the host immunologic response in relation to occurrence, persistence, and descent of these nodules.

10. Methods of treatment for filarial elephantiasis in China

Z Hui Jun, Department of Filariasis, Guizhou Provincial Institute of Parasitic Diseases, Guiyang, China

Four methods of therapy for filarial elephantiasis have been applied in China with an effective range of 30.6%-77.4%. In order to examine the mechanism of heat and bandage treatment, lymphangiography, 131I-HSA clearance test, and radioactive isotope scanning of regional lymph nodes were carried out in a few patients before and after treatment, verifying recovery of the damaged lymphatics and reestablishment of lymphatic drainage.

On the basis of our practice, it is recommended that combined therapy including bandaging, softening of the tissues of the afflicted legs with heat, filaricidal treatment, and control of secondary infection is most effective.

11. Indications and success of surgical approaches to filarial elephantiasis

S Jamal, Thanjavur Medical College, India

Inguinal lymph nodo-venous shunt resulted in 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 were also required to maintain the reduction. In follow up over 15 years the shunt maintained patency as evidenced by clinical observation of rapid diminution in the size of recurrent swelling by simple elevation leaving a fold of reduced skin for further excision. The Charles procedure should be avoided for limb filariasis in India.

12. Immunological profile of patients with lymphatic obstruction vis-a-vis other manifestations of filariasis

WF Piessens, Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts

The thesis that variation in immune responses to parasite antigens among exposed individuals determines the clinical outcome of infections with lymph-dwelling filariasis 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. Too little 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 allows evaluation of host-parasite interactions during the early phase of infection and further establishes causal relationships between immune responses and clinical outcomes. Early results of a study on immigrant populations can be summarized as follows:

Antibodies to microfilarial somatic antigens are detected 6-12 months before demonstration of sensitization of T lymphocytes by the in vitro assay of antigen-induced lymphatic 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. In contrast, filarial antigen-specific immune suppression is common among native residents of endemic areas but rare in recent immigrants. Whether these observations correlate with the ultimate outcome of filarial infection remains to be determined.

13. Immunoregulation in man and its relationship to lymphatic pathology in filariasis

TB Nutman, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

The manner in which the human host responds immunologically to filarial parasites is clearly crucial 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, though genetic, environmental, cell-mediated, humoral, and parasite-derived factors have been variably implicated. While filarial and other parasite antigens have been shown to be 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 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-, and T-cell subset responsiveness to parasite antigen, whereas those with the asymptomatic form of the infection show parasite-specific anergy in vitro. This lends further credence to the hypothesis that the host immune response is implicated in the pathogenesis of the chronic lymphatic form of filariasis. Finally, the levels of circulating immune complexes are elevated and the levels of circulating parasite antigen are negligible in patients with chronic lymphatic obstruction. This finding again suggests that the immune response—manifested by increased antigen-antibody complexes and the inability to lower parasite antigen—is in part responsible for the chronic obstruction seen in lymphatic filariasis.

14. Pathogenesis of lymphatic lesions in Brugia pahangi infected jirds

TR Klei, Department of Veterinary Microbiology and Parasitology, Louisiana State University, Baton Rouge, Louisiana

The kinetics of the granulomatous inflammatory response within the lymphatics of B. pahangi infected jirds was measured over a 180-day period after single inoculations of 50 B. pahangi L3 and the results compared to that of jirds receiving 4 or 8 similar inoculations over the same time period. Responses of antibody, lymphatic transformation, and pulmonary granulomata to crude B. pahangi antigen extracts were measured. The results indicate that: multiple infections do not produce a protective resistance to infection in jirds; in general, peak numbers of lymph thrombi and renal lymph node sizes occur between 48 and 180 days post-infection and decrease thereafter; modulation of the intralymphatic granulomatous response corresponds to the pulmonary granulomatous response and trends in lymphocyte transformation responses but not to circulating antibody titers. These findings indicate that a distinct sequence of responsive states occurs in jirds. The modulatory responses are not overcome by multiple infections. Thus, single subcutaneous induced infections at appropriately selected times should be useful for further study of the pathogenesis of B. pahangi induced lesions.

15. Observations of lymphatic pathology in nude mice parasitized by Brugia spp.

AC Vickery, KH Albertine, JK Nayar, College of Public Health, University of South Florida, Tampa, Florida.

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 lymphedema and skin changes consisting of ulcers, fissures, or hyperpigmentation develop in some mice 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.

Histologic examination of parasitized lymphatics reveals dilation and tortuosity in the absence of luminal or interstitial infiltrates and physical blockage. However, immunologic reconstitution of nude mice harboring adult B. malayi results in 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 harboring 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. Further, the susceptibility of nude mice to selective adoptive immunologic reconstitution suggests that mechanisms of adult worm killing can be characterized.

16. Limb edema in Brugia infected dogs

B Hammerberg, Department of Microbiology Pathology, and Parasitology, School of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina

Eighteen dogs sustained moderate to high microfilaremia for 18 or more months after a single large infective dose of Brugia pahangi. Upon reinfection of five very low or amicrofilaremic dogs (24 to 66 months after original infection), three did not redevelop a sustained microfilaremia, but two of these three developed limb edema. These three dogs had markedly increased antibody reactions with two bands of antigens (52,000 and 58,000 daltons on SDS-PAGE) from Brugi adult products and adult homogenate extracts. This antibody response was not detected in dogs with high microfilaremia at the time of reinfection.

Neither chronic nor transient limb edema in reinfected dogs was associated with occlusion of lymph ducts in the infected limb as shown by xeroradiographic lymphangiography. This finding suggests that local pathophysiologic mechanisms, in addition to lymph duct occlusion, plays a role in the development of peripheral edema.

By contrast, one of five dogs given a high dose of infective larvae in the paw developed chronic lymphedema starting at 12 months postinfection, associated with lymph duct occlusion. This dog had the highest microfilaremia of all dogs studied. Other dogs given large distal limb infections showed transient episodes of limb edema, associated with duct occlusion, but edema subsided after "new" lymph ducts bypassed the nonfiltering popliteal node.

The basis for lymph duct occlusion is complicated by findings of a high antibody titer in the dog with lymphedema against two antigens (50,000-55,000 daltons) from mf products. Preliminary results demonstrate that the only other dog with a response to these antigens was the amicrofilaremic reinfected dog that developed chronic limb edema.

These conclusions, drawn from in-depth longitudinal studies on experimental infections, are deemed preliminary, because of the limited number of hosts monitored. Nonetheless, the pathogenesis of filarial edema appears to derive from more than one factor. Although we have not reported on the role of monocyte/macrophage and lymphocyte function just now beginning to be studied, the size and accessibility of infected and noninfected limb lymph ducts for cannulation can be exploited in studies concerned with the local production and action of monokines and lymphokines.

17. Experimental lymphatic dysfunction caused by Brugia malayi

A Ewert, Department of Microbiology, University of Texas Medical Branch, Galveston, Texas

Domestic cats and patas monkeys can be infected with Brugia 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 edema 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 technique.

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, 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 filaria remained within the regional lymph vessels of the hind legs. Edema developed within a month of infections 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 suggested that vesicles in the cytoplasm of endothelial cells lining the lymph vessels may play a role in lymph transport. Although gross edema was not evident in infected monkeys, lymph flow alteration was demonstrated by lymphoscintigraphy.

18. Lymphatic and splenic pathology of Brugia malayi infection in the leaf monkey (Presbytis spp.)

JW Mak, Institute for Medical Research, Jalan Pahang, Kuala Lumpur, Malaysia

The non-human primates Presbytis melalophos and P. cristata are highly susceptible to experimental infection with subperiodic Brugia malayi. All animals infected subcutaneously with about 200-300 larvae over the ventro-medial aspect of the thigh and followed for more than six weeks had microfilaremia. Geometric mean microfilarial counts rose rapidly during the first month and then tended to plateau by the fourth month. Peak counts were in excess of 1600 microfilariae/ml. Developing worms were mainly found in the sacral, para-aortic lymph nodes and vessels and thoracic duct (90%) while the other 10% were in the inguinal, popliteal lymph nodes and vessels and other sites. Adult worms were evenly distributed in the sacral, para-aortic lymph nodes and vessels and the thoracic duct (45%) and in the inguinal lymph nodes and associated vessels (45%), with the remaining 10% scattered in other areas. Marked inflammatory

response and thrombosis were seen around dead worms whereas live worms induced less severe derangements primarily dilated lymphatics. In 90% of microfilarial animals, gross and microscopic granulomatous changes were seen in the spleen. The intensity of these reactions were related to the rate of decline of microfilaremia, suggesting that the spleen played an important role in destruction of microfilariae.

19. Immunology of Bancroftian filariasis in the leaf monkey, Presbytis cristata

JR Campbell, U.S. Naval Medical Research Unit No. 2, Jakarta Detachment, APO San Francisco, California

This three-year project studied cellular and humoral immune responses in Presbytis cristata monkeys experimentally infected with Wuchereria bancrofti. Animals were given subcutaneous injections of 250 third-stage infective larvae (L3) of W. bancrofti in the left inguinal region. The L3 were injected either as a single dose of 250 parasites or as ten injections of 25 parasites each. Blood was taken for sera and cells at various time points before infection, during the pre-patent 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. bancroftian antibody titers. Specific antibody titers were measured by ELISA, using excretory-secretory antigens from cultured L3 (L3-ES). It is possible that the animals make antibody against worm components other than the L3-ES antigens, and various other fractions from L3 and adult worms are currently being tested. Although displaying normal concanavalin A and pokeweed mitogen responses, lymphocytes from a maximum of 25% of the infected animals responded to any of a variety of W. bancrofti antigens by lymphocyte transformation assay (LTA). 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. The other 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.

J.W. Kazura, Division of Geographic Medicine, Case Western Reserve University, Cleveland, Ohio, presented a paper on the relationship of microfilarial status to lymphatic disease in Papua, New Guinea.

FUTURE DIRECTIONS

Important issues requiring further study identified by the who/tdr/fil scientific working group on pathology and immunopathology of lymphatic filariasis.

E Ottesen, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Washington, DC

I. Pathogenesis

A. Clinical

Since it is unclear what clinical stages individuals with filariasis must go through to progress to elephantiasis, it is necessary that longitudinal or other studies to address this issue are undertaken. Also, there is no generally accepted classification scheme used for characterizing patients with lymphatic obstruction due to filariasis, and such must be developed. Use of the recently formulated classification scheme adopted by the International Society of Lymphology should be evaluated for its usefulness in filariasis.

B. Lymphologic

A clear definition of the lymphatic-related changes in filaria-infected individuals is necessary. This definition would include the sites of actual obstruction induced by both Brugia and Wuchereria filariae as well as analysis of the cellular and humoral constituents of lymph from affected individuals. Such studies would require use of techniques for the direct sampling of lymph as well as lymphoscintigraphy and other non-invasive techniques to evaluate lymphatic function. Greater efforts should also be made to develop a panel of tests to assess the integrity

and function of the lymphatic system and to disseminate more widely information about these techniques and their usefulness.

C. Immunologic

Greater attention should be paid to analyzing and defining the specific antigens responsible for initiating immune responsiveness and immune tolerance in patients with various clinical expressions of lymphatic filariasis. The use of cloned antigens should be especially helpful in this regard. Studies of the differential susceptibilities of individuals to infection and disease must be undertaken with the hope of distinguishing among immunogenetic/immune responsiveness factors, in utero sensitization or "tolerization," and environmental factors determining the outcome of human exposure to the parasite. Immunomodulating factors (including circulating antigens and circulating immune complexes) should be analyzed in greater detail. Findings made by workers in one area studying the local species or strain of parasite should be re-examined in other areas where different parasite species or strains exist.

D. Parasitologic

Further effort to identify parasite sub-species or strains is necessary, as is the correlation of such differences with any immune response or immunopathogenic differences seen in populations with these different parasite strains.

II. Experimental Animal Models

Excellent models now exist for studying filarial lymphostatic disorders including elephantiasis in nude mice, jirds, ferrets, cats, dogs, and leaf monkeys. Many of these models have been only recently developed (e.g., Wuchereria bancrofti in Presbytis and Brugia in both nude mice and ferrets). All need further exploration to define the degrees of similarity to the infections in humans in order to indicate which models are most relevant for studying the pathogenesis of lymphatic obstruction in humans.

Attention also needs to be directed toward developing models for the acute adenolymphangitis seen in humans (e.g., the role of immune responses, bacterial super-infection, developing larvae, and the direct effects of worms and worm products).

To understand better the mechanisms leading to chronic lymphatic obstruction, the physiologic functional parameters of the lymphatic system should be evaluated in these models in order to compare them with similar observations on the lymphatics of humans. Further, the important elements in developing lymphatic obstruction (whether directly worm-induced or host immune-induced) must be determined. The nude mouse model with Brugia malayi may be especially valuable in this regard. The role of endothelial proliferation in the development of lymphatic lesions and the mechanisms underlying this "lymphangiogenesis" should be invaluable.

III. Therapy

While there is a reasonably standardized approach to treating filarial infections, there is no uniform approach to treating the various pathological consequences of filarial lymphatic obstruction. Partly, this situation results from the lack of controlled studies comparing different treatment regimens, but it is also clear that many lymphatic obstructive complications (particularly elephantiasis) are reversible. Some of the particular issues that must be addressed are:

A. Elephantiasis

1. The efficacy of diethylcarbamazine (DEC) in reducing elephantiasis and hydrocele
2. The effectiveness (i.e., short- and long-term follow-up) of surgery (particularly nodo venous shunts with excision of excess tissue) to reverse elephantiasis
3. Determination of the "active principle" in mulberry leaves and other indigenous drug treatments of elephantiasis
4. Effectiveness of benzopyrones, steroids, and other anti-inflammatory agents in alleviating elephantiasis
5. Effects of anti-microbials in halting or slowing the progression of disease
6. The role of physiotherapy and compression bandages in the management of elephantiasis in filarial endemic areas
7. The value of a primary health care approach to decreasing filarial fevers and elephantiasis through the use of readily available DEC

B. Hydrocele

1. The effectiveness of DEC or other non-surgical approaches to reduce hydrocele
2. The optimal surgical approach to this lymphatic obstructive complication

C. Chyluria

1. The effectiveness of DEC alone in reversing chyluria
2. The relative values of current surgical procedures (e.g., ligation of renal lymphatics and nephropexy) and the use of sclerosing agents (such as silver nitrate) injected into the lymphatics to stop chyluria
3. The usefulness of dietary fat restrictions to minimize the metabolic consequences of chyluria