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FILARIASIS: PROBLEMS AND CHALLENGES*

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Being President of the Society during the past year has been an enriching experience and I thank you for the privilege and opportunity to serve. My special thanks go to Dr. John Scanlon who shoulders the work of the Society so effectively.

The last time I addressed the entire Society[1] was in this city in 1972 at a joint meeting with the American Society of Parasitologists. I discussed the relationship of government, parasitology and tropical medicine and ended with an assessment of funding for research for these fields. In reviewing the history of extramural funding by the National Institutes of Health for filariasis research it was interesting to note that in 1965 only two grants existed for the total sum of $15,150. Seven years later, largely due to the efforts of the U.S.-Japan Cooperative Medical Science Program, the number of funded grants had increased to eleven for a total sum of $314,975.

I do not intend to review the problem of funding for filariasis research in this address. Analyses of funding for research in tropical medicine and in parasitology, including the filariases, were made in recent years by Cook[2] and Delappe.[3] A few weeks ago a detailed report on funding for tropical disease research was released by the Office of Technology Assessment of the United States Congress.[4] I recommend this publication to you not only for the coverage of this aspect but for an important overview of the status of biomedical research and related technology for tropical diseases. It indicates in a summary table of combined annual funding by United States sources and by the World Health Organization that the filariases receive 3% of the total available funds for research and training in tropical diseases. As was pointed out by Dr. Cook,[2] comparison of research support for parasitology with that for other areas of biomedical research such as cancer and heart disease does not fare well, considering the harsh impact of tropical diseases on world populations.

However, even a cursory perusal of the literature of the last 15 years indicates that activity in filariasis research has not only increased in quantity but more importantly in scope and in the application of new research disciplines. As has happened in other fields of biomedical research, the newer technologies and analytical approaches of immunology and molecular biology are beginning to be applied more rigorously to biological and medical aspects of the filariases with interesting implications for the future. Laboratory studies and field operations in endemic areas appear to have increased. Some progress is evident in the control of onchocerciasis in limited areas of Africa. But the massive problem of the endemic filariases, particularly that of lymphatic filariasis, tropical pulmonary eosinophilia and onchocerciasis, for the most part remains unyielding in the vast areas of the tropics and subtropics. Discouragingly, rapid urbanization is occurring worldwide. Lack of facilities for the proper disposal of sewage and waste is expanding the vector breeding sites not only in the newly-created city slums but also in the rural areas which more and more come under the domination of the towns. Populations of humans as well as vector populations are increasing at an astonishing rate, creating new hordes of susceptibles. Coupled with the flair of *Culex quinquefasciatus* to develop resistance to insecticides, and the lack of effective drugs without toxic side effects, there is little basis for optimism at this point in time. The complex ecological interplay of humans, vectors and environment that gives impetus to transmission may yield ultimately to continued efforts in the laboratory and field, but only if support for basic and applied research is available in adequate amount and expended in an intelligent manner. Our ignorance is fairly profound and our efforts groping.

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All is not bleak, however. Excellent reviews have recently appeared on significant advances being made in research on chemotherapy,[5] on immunological aspects of lymphatic filariasis and onchocerciasis[6] and on epidemiological, vector and control aspects.[7] Consequently, for the brief time available, I will only touch upon these matters and instead concentrate on a few other interesting problems which I believe are of importance to our understanding of the filariases.

OXYGEN AND FILARIIDS

The first part of my talk will be devoted to the interaction between oxygen and filariids. A discussion of oxygen requirements leads to a consideration of a multiplicity of important matters in the biology and host-parasite relationships of these parasites. It serves as a mechanism to introduce for discussion a variety of important problems, for example: 1) our inadequate understanding of the physico-chemical and nutritional factors that are required by filariids for growth, molting, differentiation and reproduction in vitro; 2) our lack of information about the detailed nature of the tissue and fluid microecological milieu which provides the intimate habitat for the various species of filariids, emphasizing the great gaps in our understanding of the pathophysiology of the host-parasite relationship; and 3) the problems and possible misinterpretations that may arise in biochemical, immunological and drug evaluation studies conducted using currently inadequate in vitro procedures.

Culture systems

One of the pressing needs in filariasis research has been the development of in vitro cultivation systems that would permit the normal growth and maintenance of all stages in the life cycle of filarial worms. For example, the availability of such culture systems would provide new and important approaches to the study of those factors which regulate and control developmental processes. Immunological studies would be enhanced in many ways. Stage-specific antigens could be harvested and a variety of investigations studying the arms of the immune effector systems could be critically examined under physiologically appropriate conditions. Screening of drugs and the study of the mechanism of drug action against particular stages would similarly become much more reliable and significant. These approaches have been commonplace in microbiology and protozoology for decades and defined culture systems have been of inestimable value to these fields. Such types of in vitro studies as I have mentioned, of course, have been performed in the past with various filariid species. Of necessity, however, they have been short term experiments using media and physico-chemical conditions we now know to be inadequate, with the parasites studied being metabolically stressed and essentially in various stages of dying during the period of in vitro maintenance.

Until fairly recently the number of studies in which attempts had been made to culture filariids in vitro were discouragingly few. In 1970, I reviewed the literature[8] and found that a total of only 43 papers had been published on this subject in approximately 60 years, the first appearing in 1912. Almost all were concerned with survival and maintenance in vitro; no significant growth or development of the worms was reported. Most of the studies were conducted with microfilariae, a few with adult worms and in only four investigations were attempts made to obtain growth of third stage larvae from the arthropod vector. In the ensuing years, however, research on all aspects of filariasis has increased considerably and with it a renewed interest in cultivation as an experimental tool. Some encouraging progress has been made and for the first time significant growth and differentiation in culture of several species of filariids have been reported.

These studies are beginning to provide us with an insight into some aspects of the biological processes of these parasites. They have also posed intriguing questions about important facets of the host-parasite relationship, hitherto unsuspected. As examples, I would like to discuss some information derived from work we have done and results reported from other laboratories.

Oxygen requirements of parasitic protozoa and helminths have been studied for decades, particularly with regard to low oxygen tensions and so-called conditions of anaerobiasis. It is apparent that exceedingly few species of parasites are strict anaerobes. The pioneering work of Trager and his colleagues[9, 10] demonstrated unequivocally the importance of a relatively low oxygen tension for the successful cultivation in vitro of *Plasmodium falciparum*. Interesting findings regarding the oxygen relationships of *Entamoeba*

histolytica also have emerged from the quantitative analytical investigations of Gillin and Diamond.[11] Studies of the effects of the gas phase on schistosomes and parasitic nematodes in culture also have been conducted but the results have been difficult to interpret because information on the actual oxygen tensions present in the culture system has not been provided.

In studies conducted by Dr. Eileen Franke in my laboratory on the cultivation of *Dipetalonema viteae,* we reported the development of third stage larvae from the arthropod vector to young adults in a chemically defined medium supplemented with serum.[12–14] Of critical importance was the gas phase, a relatively low oxygen tension being required in order for growth, molting and differentiation to occur. Surprisingly, the window for the required partial pressure of oxygen was found to be relatively narrow. Development of the larval stages occurred only between a partial pressure of oxygen of approximately 30 to 50 mm Hg. At higher and lower oxygen levels development was inhibited. *Dipetalonema viteae* thus appears to be a microaerophilic organism, at least in its mammalian cycle.

Using essentially the same medium and low oxygen tension, Dr. Vishnu Sneller reported at last year's annual meeting of the Society that adult worms of *Brugia pahangi* could be maintained for 3½ to almost 7 months. Microfilariae that were collected after approximately 3¾ months of culture developed to third stage larvae in mosquitoes.

These investigations suggest the importance of quantitatively evaluating the oxygen tensions required in cultures established with filariids of humans and of other experimental animal species. In most such previous studies, mixtures of air/CO_2 have been used with discouraging results.

That filariids appear to have the capacity to respond to very small differences in oxygen tensions present in a host is suggested by the interesting studies of Hawking[15] on the mechanism of microfilarial periodicity of *Wuchereria bancrofti.* The nocturnal migration of the microfilariae appears to be dependent upon the difference in oxygen tension that exists between pulmonary arterial and venous blood. Microfilariae accumulate in the pulmonary vasculature when the venous-arterial difference in oxygen tension is 55 mm Hg or greater. A decrease in the venous-arterial oxygen tension to approximately 44 mm Hg or below results in the migration of the microfilariae to the peripheral blood. This ability of microfilariae to titrate relatively small changes in pO_2 and to respond behaviorally suggests that developing larval and adult stages may also have the capacity to do so. Experimental studies conducted in vitro to examine these phenomena should be quite rewarding. The sensory receptors that respond to oxygen are not known. It is possible that the amphids and phasmids may function as chemoreceptors for the gas. How the circadian rhythms of other microfilarial species are entrained into the physiological rhythms of their hosts is far less well understood. The information that has appeared in recent years on mammalian circadian rhythms may provide the physiological and biochemical basis for the underlying host-parasite relationship. The location of the circadian pacemaker in the mammalian brain driving the rhythm of melatonin synthesis[16] and the integration of function of the retina and pineal gland provide an experimental tool to explore this interesting problem.

In vivo relationships

It is instructive to examine the information available concerning the oxygen tensions in tissues and body cavities that serve as habitats for various genera and species of filariids. The types of niches in mammalian hosts occupied by filariids range widely: subcutaneous tissues for *Onchocerca volvulus, Dipetalonema viteae* and *Loa loa*; the pulmonary cavity for *Litomosoides carinii,* the abdominal cavity for *Setaria* species; lymphatic vessels for *Wuchereria bancrofti* and the *Brugia* species and blood vessels for *Dirofilaria immitis.* The oxygen tensions of all of these habitats are relatively low, being in the neighborhood of approximately 40 mm Hg or lower.

Although the oxygen tensions of tissues, body cavities and body fluids have been studied by many investigators, much of the recent information is derived from studies of tumorous and cancerous growths, wound healing and cardiac vascular physiology. The sophisticated techniques utilized by investigators in these fields such as that of colpophotography[17] and stop-motion microcinematography[18] could be of considerable value in studies of the vascularization and gaseous microenvironment in the immediate vicinity of larval and adult stages of filariids. No detailed studies have been made to follow the vascular changes that occur over time in the

pathogenesis of filarial infections, particularly of the microcirculation in the immediate vicinity of developing and adult worms. In onchocerciasis, young nodules may show capillary neoformation. Ultimately, however, vascular degeneration occurs accompanied by fibrotic changes of an avascular nature in the onchocercoma and skin.[19, 20] In lymphatic filariasis in which obstructive lymphangitis, stasis of lymph flow and thickening of the walls of the lymph vessels occur, it appears possible that oxygen tensions in the immediate vicinity of the filariids may be quite low. Detailed study of changes in the vascular architecture in the filariases relative to local oxygen tensions could provide important background information regarding oxygen requirements of filariids for growth, development and survival in vivo, particularly if coupled with in vitro investigations. How these parameters are modified by chemotherapy, often with accompanying inflammatory responses, is not understood, particularly as they may affect the viability of metabolically injured parasites.

Oxygen and mammalian cells

Much information on the oxygen tensions required by a variety of mammalian cell types growing in vitro has become available in recent years. Such studies appear to have considerable relevance to cultivation attempts with particular groups of parasites and offer a model framework for future investigations. Some of the results obtained from studies on the relationship of mammalian cells to oxygen tension in vitro may be summarized as follows:

1. Both neoplastic and non-neoplastic cells have equal or better plating efficiencies at low oxygen tensions (1%–3% O_2) than under a gas phase containing air (20% O_2).[21, 22]

2. Oxygen inhibition of growth acts directly on the cells and not necessarily by destroying some essential medium component.[22]

3. The mechanism of oxygen toxicity is not clearly understood and is complex. An attractive hypothesis is that reactive oxygen radicals formed from reductive intermediates of oxygen during normal cellular metabolism damage the cells.

Oxygen and filariid energy relationships

Obviously, metabolic differences exist between mammalian cells that have a functional Krebs cycle and adult filariids that rely on glycolysis for energy production. Many species of filariids, such as *Brugia pahangi, Dipetalonema viteae, Dirofilaria uniformis* and *Onchocerca volvulus,* are exclusively homolactate fermenters. This aspect has been investigated in detail for adults of *B. pahangi* and *D. viteae* using isotope and aerobic fermentation balance studies.[23] These two species observed in vitro over a 24-hr period continued to move actively in the absence of oxygen. However, movements of *L. carinii* rapidly ceased in a medium depleted of oxygen. This species, in addition to requiring oxygen for movement, also differs from *B. pahangi* and *D. viteae* in that it accumulates acetate, acetoin and unidentified compounds in addition to lactate. *Litomosoides carinii* does not appear to possess an actively functioning tricarboxylic acid cycle to account for its utilization of oxygen[24] although some reports to the contrary have recently appeared.[25, 26] Instead, it apparently requires oxygen primarily for the oxidative decarboxylation of pyruvate to acetate and CO_2, presumably with the production of ATP.

If oxygen is not required by filariids for energy production through a functional Krebs cycle, an anomalous situation exists with regard to the nature of their mitochondria. These organelles are highly cristated in all three species,[24] such a condition usually implying an active TCA cycle. This is an interesting problem requiring resolution since it has important implications with regard to potential sites and mechanisms of drug action.

Collagen synthesis

It is also necessary to reconcile the seemingly contradictory fact that although molecular oxygen presumably is not required for the movement of *B. pahangi* and *D. viteae* adults, it is required for the developmental processes in the growth and differentiation of third stage larvae of *D. viteae* to the adult form in vitro. Observations of this nature suggest that oxygen, although possibly not required for energy-yielding pathways, may be needed for other metabolic processes. For example, collagen synthesis, which is essential to nematode cuticle formation, is an oxygen-dependent process in which proline-4-monooxygenase converts the proline in protocollagen to hydoxyproline.[27] It is of interest that the enzyme in adult *Ascaris* is partially inhibited at oxygen concentrations exceeding 1%–5%.

However, inhibition does not occur in the aerobic egg, suggesting the existence of isozymes.[28] Whether isozymes of this hydroxylase occur in the larval and adult stages of filariids requires investigation.

Ecdysteroid synthesis

Another oxygen-requiring process that may be linked to filariid development is the synthesis of ecdysteroids such as ecdysone. This steroid functions as a molting hormone in insects and is of critical importance to the developmental process. Ecdysone and ecdysteroids have recently been isolated from adult *Dirofilaria immitis* taken from the dog host[29] but the origin of these hormones was not determined. Studies done in my laboratory on the larval cultivation of *Nippostrongylus brasiliensis* under axenic conditions and in medium of known sterol composition have provided strong evidence supporting the concept that nematodes are capable of synthesizing ecdysteroid-like compounds from cholesterol in their diet.[30] It is important to recognize that in insects synthesis of ecdysteroids from cholesterol is dependent upon the presence of molecular oxygen and presumably this would also hold true for nematodes.

Much work is now required not only to determine the presence and function of these ecdysteroids in filariids and other parasitic nematodes, but also their mode and specific site of synthesis. As has been found to be the case with insect species, considerable differences in the ecdysteroid biochemistry of nematodes should be anticipated. Of considerable interest in this regard has been our finding that azasteroids (25-azacholestane and 25-azacoprostane) are extremely potent inhibitors of nematode development, disrupting growth, molting and reproduction,[31-33] comparable to that which occurs to nematodes grown in the absence of cholesterol. It is possible that these compounds act as inhibitors interfering with the incorporation of cholesterol into membranes. For example, the addition of azasteroids to axenic cultures of the larval stages of *N. brasiliensis* results in severe repression of development, abnormal molting and degeneration of the intestinal cells. However, azasteroids may also be functioning as inhibitors of ecdysteroid synthesis comparable to their action in insects.[34] These findings suggest new targets for the development of chemotherapeutic agents for nematodes and the use of azasteroids as metabolic probes in the study of cholesterol metabolism in these organisms. It will be of interest to determine the requirement for oxygen in this regard.

Oxygen toxicity

It is apparent that the need for oxygen by nematodes is quite diverse and complex. In the case of filariids such as *D. viteae,* two completely different mechanisms appear to be operative. At quite low oxygen concentrations the limiting aspect may be insufficient oxygen to support synthetic and metabolic processes required to sustain the life of the organism. At relatively high oxygen tensions, however, the mechanism of oxygen inhibition very probably is due to its toxicity. Explanations to account for the oxygen toxicity that occurs in many species of organisms have come from a variety of sources. Some of the hypotheses that have been proposed suggest that: 1) oxygen inhibits metabolism by oxidizing SH groups of enzymes; 2) oxygen causes the peroxidation of lipids and the resulting lipid peroxides inhibit enzyme function and affect membrane integrity; and 3) oxygen radicals formed during normal metabolism damage cells.

Although one or a combination of these factors may be operative in filariids, virtually no experimental data exist to clarify the matter. Much work remains to be done to determine the presence and types of oxygen intermediates that may arise during metabolism. Hydrogen peroxide has been reported to be toxic to *L. carinii.*[35] However, it is not known whether scavenger enzymes such as catalase, superoxide dismutase and glutathione peroxidase are formed to remove reactive oxygen intermediates. Such investigations could be of considerable importance in clarifying the toxic nature of oxygen for filariids. Oxidant stress on *Plasmodium falciparum,* as discussed by Scheibel and Adler[36] and Allison and Eugui,[37] may seriously injure the parasite.

Another aspect of the possible interaction of filariids with reactive oxygen intermediates arises not from their endogenous metabolic synthesis of these compounds but rather from the generation of the oxidants by the cells of the immune response. Of interest in this regard are the recent findings of Kazura and Meshnick[38] concerning the disparity in the susceptibility of the different stages in the life cycle of *Trichinella spiralis* to

killing by neutrophils and eosinophils. Their observations indicate that newborn larvae were more susceptible to being killed by oxidants than were muscle larvae or adults. It was hypothesized that adults and muscle larvae may be more resistant to killing by leukocytes than newborn larvae because they contain scavenger enzymes enabling them to resist oxygen-mediated damage. Comparable studies of this type with filariids are needed to determine whether the oxidative intermediates of neutrophils and eosinophils, which are known to severely damage the cuticle of various stages of the parasites, may be nullified to any degree by the presence of oxygen scavengers in the worm. Forsyth et al.[39] have hypothesized that albumin which is adsorbed in vivo to microfilariae of *Onchocerca gibsoni* may be acting as a local inhibitor of eosinophil peroxidase-mediated oxidative damage. Bovine serum albumin is a known scavenger of hypochlorous acid which is generated by the reaction of eosinophil peroxidase with H_2O_2 and Cl^- and which is capable of killing newborn *T. spiralis* larvae.

Carotenoids and vitamin A

Other chemical factors in the tissue environment of filariids may also serve to protect them from oxygen damage. Vitamins, such as vitamin E and ascorbic acid, are important antioxidants in vivo and their effects on larval development using in vitro cultures should be assessed. In this regard, Stürchler et al.[40] have reported that the retinol (vitamin A alcohol) concentration in tissues of *O. volvulus* is very high, averaging eight times higher than the skin of infected individuals. Carotene and retinols have a multiplicity of functions and it will be of interest to investigate these in filariids.

Carotenoids, although not extensively studied, have been reported to be present in a variety of parasitic helminths: in the larval cestode *Schistocephalus solidus,*[41] adult trypanorhynch cestodes[42] and in *Foleyella furcata,* a filariid parasite of chameleons.[43] Comley and Jaffe,[44] stimulated by the observations on the high concentration of carotene and vitamin A in *O. volvulus,* have studied the biochemistry of these compounds in *Brugia pahangi.* The adult filariid took up and incorporated β-carotene and retinol and the in vitro conversion of β-carotene to retinol was demonstrated. The retinyl phosphate formed from retinol was considered to be potentially involved in glycoprotein synthesis.

It is possible, however, that β-carotene and retinol may also have another function in filariids which is worthy of investigation. β-carotene, a purported anticancer agent, has been believed to have antioxidant action of a radical-trapping type. More recently, in vitro experiments have demonstrated that it belongs to a previously unknown class of biological antioxidants.[45] It exhibits good radical-trapping antioxidant behavior only at partial pressures of oxygen significantly less than 150 torr, the pressure of oxygen in normal air. The low oxygen pressures in which it acts as an antioxidant are found in most tissues under physiological conditions, which would include the various habitats of filariids. At higher oxygen pressures β-carotene loses its antioxidant activity and shows an autocatalytic pro-oxidant effect, particularly at relatively high concentrations.

The results obtained by Burton and Ingold[45] have demonstrated that β-carotene has the potential to play an important role in protecting lipid from peroxidation in vivo. The chain-breaking action of β-carotene complements that of vitamin E since β-carotene is effective at low oxygen concentrations and vitamin E is effective at high oxygen concentrations. As pointed out previously, filariid habitats have low partial pressures of oxygen and it may, therefore, be anticipated that retinoids would exhibit antioxidant properties in filariids such as *O. volvulus, Brugia pahangi* and *Foleyella.* Studies to examine this relationship should be of considerable interest.

Another important function of retinoids, that involved in the regulation of gene expression, has recently been reported. Retinoic acid, as a naturally-occurring metabolite of vitamin A, has been known to modify the proliferative rate and differentiation of a variety of normal and transformed human cells. Recent studies[46, 47] suggest that retinoic acid and serum retinol-binding protein can directly regulate gene expression of mouse macrophages and human promyelocytic leukemia (HL-60) cells and specifically induce the synthesis of tissue transglutaminase. Such regulation of gene expression as a property of retinoic acid and its binding protein may have important implications with regard to the growth and differentiation of *O. volvulus* and other filariids which concentrate the vitamin to a marked degree. In

vitro culture should provide a valuable tool to assess their direct effect on the actual growth and differentiation of larval and adult stages.

Vitamin A deficiency, unfortunately, is an all-too-common occurrence among human populations in areas endemic for filariasis. Laboratory studies on the association of vitamin A and filariasis obviously should be integrated with clinical investigations in the field. Stürchler et al.[40] posed the pertinent question of whether onchocerciasis depletion of retinol in host tissues might be a contributory factor in the pathogenesis of corneal opacities, localized dermatitis (sowda) and other symptoms occurring in both clinical onchocerciasis and hypovitaminosis A.

COBALAMINS AND FILARIIDS

Please forgive what may appear to be somewhat exaggerated speculations on the possible β-carotene-vitamin A relationship in filariids. My interest in the unusually high concentration of these compounds in these nematodes stems from my previous experience in unexpectedly having found extraordinarily high concentrations of vitamin B_{12} in a considerable variety of parasitic helminths. Unraveling aspects of cobalamin physiology, biochemistry, evolutionary distribution in helminths and its role in the host-parasite relationship has occupied much of the time of my laboratory during the past years. Interestingly enough, unlike the situation in most eukaryotic animals, vitamin B_{12} and its cobalamin derivatives do not occur in any of the filariids we have assayed. These findings correlate well with the fact that filariids are primarily lactate fermenters. The large number of parasitic helminths which contain cobalamins form propionate and do so through a cobalamin coenzyme-dependent step. This has been found to be true especially for the more "primitive" groups of parasitic nematodes such as strongylids and ascarids. The more highly evolved tissue and vascular parasites such as *Angiostrongylus* and filariids have apparently lost the ability to take up and utilize cobalamins and presumably have deleted the enzymatic pathway from succinate to propionate which requires the B_{12} coenzyme, adenosylcobalamin.

Vitamin B_{12} is an interesting molecule in that it can function in two completely different coenzyme forms. I have already mentioned the conversion of the vitamin to adenosylcobalamin which participates in an isomerization reaction in the conversion of succinyl CoA to methylmalonyl CoA. Vitamin B_{12} can also be converted to the metabolically active coenzyme, methylcobalamin, which acts as a cofactor for methionine synthetase, in the conversion of homocysteine to methionine. In the elegant work of Jaffe and his colleagues on the folate metabolism of filariids, they were not able to demonstrate the presence of methionine synthetase in *Brugia pahangi*.[48] This strongly suggests that methylcobalamin, if present, is either inactive or it does not exist in this parasite. On the basis that we have not been able to demonstrate the presence of cobalamin in this species, I believe the latter interpretation to be correct. Interestingly, we have searched for the presence of methionine synthetase and methylcobalamin in a few other genera of parasitic helminths. Although we were able to demonstrate the adenosylcobalamin form of the coenzyme in these organisms we have been unsuccessful in finding any suggestion of the presence of methylcobalamin or methionine synthetase.[49] Perhaps the ready availability to parasites of preformed host methionine accounts for this. In any event, these results suggest the possibility that in the evolution of filariids genetic deletions have occurred eliminating this arm of cobalamin metabolism.

GENETICS OF THE FILARIASES

Parasite genetics

Such studies emphasize our ignorance about the very important field of the evolution and genetics of parasitic helminths, particularly filariids. Although a considerable amount of work has been done by systematists to delineate the evolutionary patterns of filariids based on morphology and life history studies, little is known about the genetics of these organisms. This matter is of great significance to our understanding of the epidemiology and disease relationships in filariasis. In this regard, the genetics of the mammalian host and the genetics of the arthropod vector inexorably interlock with the genetics of the parasite to form a dynamic interacting trinity. These relationships unquestionably are extremely difficult to approach and analyze experimentally but the rewards could be significant in

our understanding of the complexity of the filariases.

Epidemiological data abound suggesting the genetic modulation of the filariases in nature. For example, in onchocerciasis it is well-known that the clinical expression of disease differs considerably between the Sudan-savannah zone and the West African forest. In the Cameroon, experimental data indicate that there appear to be two main *Onchocerca/Simulium* complexes, each distinct from the other and associated with a different clinical pattern. Findings such as these have important implications for research and control efforts.

Because of the considerable technical problems involved, the genetics of filarial worms remains obscure. Only a few experimental studies have been conducted, these essentially being simple crossing attempts. Hybridization experiments were done between *B. malayi, B. pahangi* and *B. patei* and crosses were obtained with various combinations of the three species.[50] Hybrid males failed to produce spermatozoa but females were fertile and produced microfilariae when crossed with males of the parental species. The strains of parasites used in these studies had been in the laboratory for different periods of time and were from different geographic areas. Comparable investigations were done more recently by Cross et al.[51] which in the main confirmed and extended these studies. The possibility was suggested that in certain areas of Indonesia, *B. malayi* and *B. pahangi* are hybridizing naturally in cats and may be transmitted to humans. The interesting observation was made that it is possible such hybrids conceivably could develop to adults without producing microfilariae, leading to occult filarial infections.

Genetic studies should be firmly rooted in chromosomal analysis. However, chromosomal cytological studies have been done for only a few species of filarial worms. Analyses of the karyotypes of *B. pahangi* and *B. malayi* revealed that both species are karyotypically indistinguishable from each other confirming their close taxonomic relationship.[52] However, chromosomal studies of Guatemalan strains of *O. volvulus* from humans and *O. gutturosa* from bovines indicate that they are karyotypically distinctive.[53] Biochemical isozyme patterns and morphological data also support these conclusions. However, some confusion exists among different investigators concerning the chromosomal patterns of human *O. volvulus* from Guatemala, Venezuela and Africa. Part of the problem seemingly relates to technical difficulties of chromosome analysis. Resolution of this problem could help interpret the clinical, vector transmission and epidemiological differences that are manifested in onchocerciasis in different areas of the world.

It is apparent that a number of approaches are required to help resolve the matter of identification of species and strains of filariids. Application of the techniques of molecular biology, isozyme analysis and immunology undoubtedly will be of great value. Monoclonal antibody procedures and the use of DNA probes are providing important new tools, but will require great care in developing specificity and sensitivity.

Genetics of vectorial capacity

Although the genetics of vectorial capacity of filariids is undoubtedly the best understood aspect of genetic modulation in the filariases, the field is still at a fairly primitive level of development and offers considerable opportunities for studies basic to understanding the host-parasite relationship. It is of vital importance to any consideration of attempts for the control of filariasis by the replacement of vector populations with filaria-refractory populations. Filariid development in the insect vector is invariably intracellular and a single host cell type is associated with each filariid species. Apparently, only ticks provide more than one cell type in an organism for the development of a filarial species. In insect vectors, however, these are exclusively either the thoracic muscle cells, the cells of the malpighian tubules or the fat body cells. Presumably, the large size of these cells, adequate to accommodate a developing larva, governs the restriction to these three host cell types. The mechanism of recognition and penetration of a specific host cell type by a migrating microfilaria in an insect is not at all understood but apparently it is not a fortuitous event. It is possible that the microfilaria recognizes and responds to specific cues and to a particular cell surface membrane component, but I am unaware of any attempts to study this matter.

Susceptibility of mosquitoes to infection with filariids is inherited, being controlled by simple sex-linked alleles in some groups, and a polygenic condition in others. Refractoriness to infection has been found to be dominant to susceptibility

but incomplete penetrance occurs.[54, 55] Different genes control the development of the various filariid species in the different host cell types.[56] Since arthropod development is fairly rigidly controlled by various hormones such as ecdysone and juvenile hormone it was anticipated that manipulation of host hormones might affect filarial development. So far, this has not proved to be the case but this aspect has not been explored in depth.

It is likely that mosquito refractory genes directly affect host cell types in which the filariids develop but the mechanism remains obscure.[56, 57] How mosquito gene regulation affects parasite intracellular differentiation at the biochemical and molecular level is one of the important challenges for future investigators. If it becomes possible with a high degree of success to culture the arthropod phase of the filarial cycle in vitro using infected mosquito cell explants, it may provide a tool with which to examine such factors experimentally.

The role of intracellular microorganisms that exist in mosquito vectors as well as in many species of filariids is quite perplexing. The precise nature of these organisms is unknown since they have not been isolated and studied in culture. It has been proposed that in mosquitoes the rickettsia-like organism, *Wohlbachia pipientis,* is the basis for cytoplasmic incompatibility.[58] It has also been suggested that the mosquito symbiont provides some necessary component for the development of filarial parasites in mosquitoes.[59]

The nature of the symbionts in filariids is also unknown, being considered rickettsia-like or *Chlamydia*-like.[60–62] Although the precise route of transmission of the symbiont in filariids is unclear, like that of the symbionts in mosquitoes it appears to be transovarial. Whether the microorganisms infecting mosquitoes may also infect their filariid parasites, or vice versa, has not been determined.

Of particular significance is the role that these intracellular organisms may play in the biology of their respective hosts. The possibility exists that they may be pathogenic in filariids. However, consideration should also be given to the possibility that a metabolic and nutritional relationship may exist between the symbionts and their filariid hosts comparable to that described for the symbionts present in some kinetoplastids and in reduviids. This would have important implications for any studies on the biochemistry, nutritional requirements and development in culture, particularly if antibiotics were used. Other issues in the relationship of filariids and their symbionts may also be of significance. It would be of interest, for example, to determine the antigenicity of the symbionts and whether their presence in filariids may prove to be confusing to immunological studies. Conceivably, they may also interfere with DNA and RNA analytical studies.

This entire matter has become highly complex and controversial and is sufficiently important to require further detailed investigation. At issue is the understanding of whether the symbionts significantly affect the reproductive biology of mosquitoes, the genetics of the vectorial capacity of filariids, as well as other aspects of filariid biology.

Genetics of the mammalian host

Specific information concerning the genetic modulation of filarial infections in mammals is still scanty. Interest in this aspect for the lymphatic filariases[63] derives primarily from correlating parasite prevalence data in particular localized human populations with clinical expressions of infection such as the asymptomatic state or symptomatic conditions such as fevers, chronic lymphatic obstruction and tropical eosinophilia. In onchocerciasis in Africa, a difference exists in the severe ocular complications of the disease in infected forest patients compared with those in the savannah. In addition, paradoxically, in the forest the number of nodules per individual is vastly more than in the savannah.[64] Obviously, nongenetic factors may play the vital role in these conditions. But the question, nevertheless, persists as to whether familial predisposition occurs and whether genetic correlates can be identified.

Clear-cut examples of the involvement of mammalian host genetic factors have been demonstrated in nematode infections in experimental animals. For example, the major histocompatibility complex (MHC; H-2 linked genes) influences the response of mice to infection with *Trichinella spiralis.* In studies evaluating potential genetic influences on susceptibility to bancroftian filariasis among Polynesians, significant familial clustering of patients with filariasis was found and the clustering was most compatible with genetic transmission of disease susceptibil-

ity. However, there was no evidence for association between individuals' HLA antigen specificities and either susceptibility to filarial infection or predisposition to any particular clinical manifestation of this disease.[63] In a similar study with two ethnic groups, Sri Lankans and Southern Indians, it was found that a heterogeneity in HLA antigen frequency existed between patients with elephantiasis and controls.[65] However, the B15 antigen was found to be strongly associated with the development of elephantiasis in both ethnic groups. To explain the differences obtained between the two studies, it was suggested that the HLA antigen is probably only a marker for some other disease-associated genes on chromosome 6 that is in linkage disequilibrium with the HLA antigen, in this case B15. The strength of this linkage may vary from population to population. It will be interesting to determine whether the association of B15 with elephantiasis is reflected in differences in immune response to the parasite.

The association between blood groups and the filariases remains controversial, to some degree because of the differences in methodology and statistics that have been applied. In a recent study conducted in India,[66] no association was demonstrated between blood groups and infection with *W. bancrofti.* Similarly, in studies on onchocerciasis in Zaire, the ABO blood groups and sickle cells were used as genetic markers. These factors could not be related directly to any constitutional predisposition to develop the lesions of river blindness.[67] However, other genetic factors could not be ruled out.

Studies on the genetic modulation of the filariases among human populations are difficult to conduct and interpret for many reasons. However, they may ultimately provide important insights into our understanding of the geographic differences in disease, pathology and immune response and the mechanisms that may be involved.

PROBLEMS AND CHALLENGES

Obviously, I have not discussed a host of problems of compelling nature vital to our understanding and control of the filariases, such as the lack of truly effective drugs having minimal side effects, the possibility of developing protective vaccines, and a multiplicity of vector and epidemiological problems. These are matters which regularly receive a good deal of attention by many groups. Instead, I have focused on a few matters that I believe are equally fundamental to our comprehension of the filariases and which require study.

Despite the importance of basic research to provide an underpinning for control efforts for the filariases, I wish to close with the proscription provided by Dr. Philip Russell[68] in his presidential address entitled "Excellence In Research Is Not Enough." Let us assume eventual success. The development of a promising vaccine or drug only signals the beginning of the long, arduous and expensive task of field and clinical trials. For example, funds to underwrite such efforts must be sought in competition with other equally pressing programs also struggling for priority. Careful planning in the endemic area of choice must be accomplished to provide an infrastructure of dedicated, well-trained local personnel capable of conducting a long range program consonant with national public health goals and budgets. These are some of the challenges for the future. I have no doubt but that many of the young members of our Society as well as others worldwide will participate in the work of the exciting years that lie ahead. The reward is good science, the sense of kindredness that shapes the human spirit and the alleviation of misery and disease for large numbers of the world's human population.

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