APPLICATION OF BIOTECHNOLOGY TO THE STUDY OF FILARIAL PARASITES AND THEIR VECTORS

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ABSTRACT

Over 200 species of filarial parasites have been described, although the life cycle and nature of their obligate intermediate arthropod vectors have been identified for only about a quarter of them. Traditional methods of studying phylogenetic relationships between closely related parasite species have utilized morphologic, biochemical and biologic characteristics, usually of the microfilarial stage. Identification of competent vectors from among complexes of sibling species, has employed similar techniques, despite the fact that differences between geographical isolates may reflect environmental rather than genetically controlled factors. Studies of the prevalence and transmission of animal, human and zoonotic filarids, so important for vector identification and control, has lead to the examination of filarial parasites at the genetic level. Genomic DNA libraries are being constructed and screened for clones which are species specific. From this work, DNA probes which can accurately enumerate larval stages in vector squash preparations, and monoclonal antibodies specific for defined filarial antigens, are being prepared. The nucleotide sequences of rRNA are also being defined. The application of these technologies to the study of filarial parasites and their vectors, promises to not only allow the construction of accurate phylogenetic trees, but also to provide the data necessary for the identification and control of the vectors of filarial pathogens of animals and man.

INTRODUCTION

Over 200 species of filarial parasites have been described, although the life cycle and nature of their obligate intermediate arthropod vectors have been identified for only about a quarter of them. Traditional methods of studying phylogenetic relationships between closely related parasite species have utilized morphologic, biochemical and biologic characteristics, usually of the microfilarial stage. Measurements of distances between anatomical features, expressed as percentages of total microfilarial length, the arrangement of caudal nuclei and presence or absence of a sheath, are useful. However, ingestion of microfilariae by the vector with progression of maturation through the larval stages, result in parasites which are very difficult to identify. A variety of biochemical techniques such as isoenzyme characterization using thin-layer starch gel electrophoresis and histochemical staining for localization of enzyme activity, have been helpful in elucidating taxonomic relationships between filariids. These methods, however, suffer from problems associated with inadequate availability and improper storage and processing of materials. Biological characteristics of filarial parasites are perhaps the

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least reliable for identification as they rely in part upon the vagaries of vector capability and host responses which are themselves subject to inherent variability.

More recently, the development of a rapid rRNA sequencing procedure has begun to provide polypeptide and nucleotide sequence comparisons for a variety of filarial and other nematode parasites[1]. If genetic distances calculated from these sequence comparisons reflect times elapsed since the divergence of the species, the separation of most of the parasite species analysed would appear to be very ancient events which may predate the separation of their mammalian hosts. Thus, it would seem that besides improving parasite identification procedures, a deeper understanding of the molecular evolution and evolutionary relationships between parasites, their hosts and vectors, may be achieved.

Identification and speciation of competent insect vectors from among complexes of sibling species is very important not only for basic biological and epidemiological studies, but also for successful vector control. This way, studies of potential vectors may be designed to target only relevant species. In general, these studies have employed techniques similar to those used for the parasites they transmit despite the fact that differences between geographical isolates may reflect environmental rather than genetically controlled factors. A commonly used technique, isoenzyme analysis[8] by starch gel electrophoresis of larval and adult stages, has resulted in the splitting of species complexes into relatively few species, probably because only a minority of the enzyme systems examined show variants with some level of diagnostic value. Also, biochemical characteristics such as enzyme variation tend to show overlap in much the same way as mor-phological markers. Interpretation is, of course, made easier when fixed alternative alleles which are electrophoretically detectable, are found in sympatric populations. However, the calculation of genetic distance, while more accurate than morphology, is not an infallible predictor of the ability of allopatric populations to interbreed when vector control measures break down.

In addition to taxonomic considerations, and perhaps of greater practical importance, has been the use of enzyme analysis in the demonstration of organophosphate resistance in several insect species. The observation of a direct relationship between the genes for organophosphate resistance and elevated esterase activity has led to the development of a simple filter paper test for determining resistance[9].

Studies of the prevalence and transmission of animal, human and zoonotic filarids, so important for vector identification and control, has lead to the examination of filarial parasites and their vectors[10] at the genetic level. Much valuable basic knowledge about these organisms is being generated. For example, analysis of the base composition of a number of filariids has shown unexpectedly low GC contents and a lack of methylcytosine in the DNA of Brugia malayi[11]. The genome size has been estimated to average $8 \times 10\ 7$ bp and the haploid and diploid chromosomal numbers of two Brugia species are n=5 and 2n=10 in both sexes[12]. Genetic evaluation using conventional techniques as well as newer biotechnology, eliminate much of the uncertainty inherent in the interpretation of secondary characteristics which may be subject to influences of the environment.

Eukaryotic DNA appears to be divided into two functional types; that which codes for proteins and that which makes up the intervening spacer regions. Much of the

spacer DNA and non-transcribed spacer regions of ribosomal genes contains families of highly repeated sequences which often are arranged in tandom arrays. This has been shown to be true for several species of brugian filariids[2,3,4].

Of particular interest, however, has been the finding that repeat sequences exist which are unique to particular filarial species, as well as sequences which are common across species lines. The isolation, cloning and use of these highly repetitive sequences to generate species-specific DNA probes for definitive identification of human and animal filariids is rapidly becoming a reality[5]. These probes which are designed to detect highly repeated DNA sequences present in all developmental stages of the parasite, are not only specific but also extremely sensitive.

Clearly, the use of DNA probes shows great potential for speciation of filarial nematodes and for determining both the vertebrate and insect host range of these species. This will be of particular importance in areas of the world where sympatric species may utilize the same vectors. The ability to monitor rates of transmission and gather data on filarial prevalence promises to be facilitated by this technology. Probe hybridization onto nitrocellulose filter squash preparations of single mosquitoes, can detect as few as one infective larva of *B. malayi*[6]. The development of non-radioactive probes could simplify their use in the field. However, these probes are not stage specific and cannot differentiate between infective larvae, earlier larval stages or microfilariae. Thus, the concommitant use of a stage-specific monoclonal antibody might be necessary when such an ability to make these distinctions is important.

Construction of cDNA or genomic expression libraries of a number of filariids is providing reagents in the form of translation products to be used in the investigation of antigens which participate in protective and pathologic immune responses in the vertebrate host[7]. This technique is proving to be particularly valuable in the case of *Onchocerca volvulus* on which research has been hampered by the relative unavailability of parasite material which must be obtained from the human host. Indeed, cDNA clones which express antigens associated with infective larvae of *O. volvulus*, which stimulate proliferation of cells from infected individuals, and which react strongly with immune sera, have been produced[13]. Cloning and expression of surface polypeptides of *O. volvulus* microfilariae, potentially relevant to vaccine development, are also in progress[14]. *Wuchereria bancrofti,* another filariid about which little is known due to the inability to obtain adult worms, would also be amenable to study using these techniques. Thus, it is now feasible to employ recombinant antigen preparations resulting from the molecular cloning of the antigen genes in experiments which formerly depended upon the preparation of antigens purified from hard-to-obtain native parasites using conventional biochemical techniques. It is difficult to underestimate the significance of the availability of large quantities of recombinant antigens for the basic characterization of filarial parasites and for the development of survey techniques, immunodiagnostic reagents and potentially immunoprophylactic measures.

It seems reasonable to expect that using the techniques of gene cloning and the development of sensitive DNA clones for hybridization, we are on the edge of an explosion of knowledge about filarial parasites and their vectors. Manufacture of recombinant filarial antigens and highly specific monoclonal antibodies will advance the development of immunodiagnostic probes

and immunoprophylactic reagents by "light years". Assessment of genetic variation between genes of related species of vectors and parasites will allow the construction of phylogenetic relationships and provide valuable insight into the co-evolution of parasites, their vectors and vertebrate hosts. In short, we are coming into a fascinating era in the study of parasitism.

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QUESTIONS AND ANSWERS :

1. Question: You mentioned the need to identify "immunodominant" antigens in order to develop effective vaccine preparations for filariasis. How would you distinguish between an antigen which is a potent immunogen, yet not protective, vs a protective antigen which is less immunogenic and how could you select such an antigen as a vaccine component ?

   Answer : Difficult problem - why I consider vaccine development to be a "long-term goal".
   Possibly - tools for T cell reactivity - i.e. screen specific T cell clones or patient T cells - rather than use sera which appear to recognize potent immunogens which are not necessary important for protection.
   To identifity protection - go back to analogous (admittedly not perfect) animal models.

2. Question: How close are we to being able to screen (infected) individual hosts for immuno-reactive T cells ?
   Is this not going to be essential for development of immunization schemes ?

   Answer : Yes - it will be essential.
   Working on procedures now - T cells from infected individuals development of T cells clones specific for Ags of interest.