CURRENT TECHNOLOGICAL APPROACHES TO THE STUDY OF TREMATODES

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ABSTRACT

Advances in biology sciences over the past decade have provided additional technology for dealing with trematode parasitoses. Although trematode diseases of man and animals in Indonesia are essentially the same as they were ten years ago, scientific methods available to deal with them have improved significantly. One can now exploit the innate ability of cells to replicate and produce biological products upon demand, manipulate the genetic make up of an organism, biologically or synthetically manufacture peptides and rationally develop drugs that target idiosyncrasies of parasites at the cellular and molecular levels. Further, one can now manage and analyze massive amounts of biological data using desk top computers. These new biological techniques and the computing ability to interpret the data generated provide parasitologists in Indonesia and elsewhere with the ability to document the economic and public health impact of trematode parasitoses and to develop new strategies and reagents for diagnosing, treating, preventing and controlling the diseases they cause. In addition, biotechnology offers university scientists and their students with additional opportunities to investigate basic and esoteric aspects of host-parasite interrelationship that are such an intriguing aspect of biology.

INTRODUCTION

Trematode parasites in Asia and the basic strategies available for their control and/or eradication have been available for decades. Parasite idiosyncrasies are identified to make them easier to diagnose, more susceptible to prophylaxsis/therapy, or succumb to environmental control measures. Schistosomiasis has been the focus for most applications of recent biotechnological developments and it is appropriately the subject of a separate review. This paper will discuss other trematodes of interest to parasitologists in Indonesia and review conventional and novel applications of new technologies that have enhanced our understanding of the basic biology of trematodes and, hopefully, will lead to their control or eradication. It should be emphasized, however, that current research efforts on schistosomiasis will provide valuable information on the biology and biochemistry of trematodes

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in general. This information will benefit subsequent studies of other trematode diseases of economic or public health importance to Indonesia.

Table 1 lists the major trematode parasitoses of Indonesia and neighbouring countries. Approximately 10 percent of recent literature on the application of new technology involved trematode parasitoses other than schistosomiasis. The minimal application of new biotechnologies to other trematode disease clearly underlines the importance of this conference. Modern biological tools, where applicable, can and should to combat other trematode diseases in Asia of economic and public health importance.

Diagnosis

The influence of molecular heterogenicity on the identification and immunodiagnosis of trematodes was summarized by Simpson (1987). Hybridoma technology has proved extremely effective in the field of immunodiagnosis. Specific and generic monoclonal antibodies have been developed to identify antigens of a variety of trematodes for use in serodiagnostic tests or as probes for specific segments of genomes. (Norden and Strand, 1984a, 1984b, Mott *et al.*, 1987; Kobayashi, 1986, Feldmeir *et al.*, 1986; Noguiera-Queiroz *et al.*, 1986; Harn *et al.*, 1985; McCutchan *et al.*, 1984; Mitchell *et al.*, 1983; Feng *et al.*, 11985; Sirisinha, 1988). Trudgett *et al.* (1988) Monoclonal antibodies, specific for tegumental antigen of *Fasciola hepatica*, were used as a solid phase immunosorbant for purification of tegmental antigen from adult liver fluke homogenates and these antigens were subsequently used in immunoassays for *F. hepatica* antibody in sheep and cattle.

DNA probes have proved both sensitive and specific in the identification of protozoan infections (Berry and Peter, 1984) and can be purchased commercially in diagnostic kits (Klausner and Wilson, 1983 and Olsiewski *et al.*, 1984). The use of recombinant DNA technology for identification of helminths has just begun, but it will prove to be a valuable tool for the purpose. Rollison *et al.* (1986) have investigated nuclear DNA, as diagnostic probe, for detection of schistosome infections. Sirisinha (1988) has identified, characterized and is attempting to clone the genes that code for candidate diagnostic protein of *Ophisthorchis viverrini* which is found in adults, metacercariae, somatic extracts and metabolic products.

Vaccines

The size of the genome varies with the complexity of the organism and the difficulty of developing rational vaccines. The genomic size of DNA viruses is around 200 thousand base pairs and protozoans have about 40 million base pairs. In contrast, helminth genomes are in the order of 200 - 400 million base pairs (Simpson *et al.*, 1982). The relatively large size of helminth genomes is one reason why hybridoma and recombinant DNA technologies have not had the impact on the development of vaccines against trematode parasites as they have had on less complicated organisms (Murray, 1987).

Fascioliasis has been the target of a limited vaccine development efforts. Various attempts to stimulate resistance against fascioliasis were summarized recently by Haroun and Hillyer (1986). Fascioliasis vaccine development has been focused on identification and characterization of antigens, particularly those antigens shared with *Schistosoma mansoni* (Hillyer, 1984). Most recent

Table 1. Trematode Parasitoses of Indonesian and Neighbouring countries.

Clonorchiasis
Dicrocoeliosis
Eurytrematosis
Echinostomiasis
Fascioliasis
Fasciolopsiasis
Heterophyidiasis
Opisthorchiasis
Paragonimiasis
Paramphistomiasis
Schistosomiasis

reports characterize tegument antigens which cross- react with surface glycoproteins of *S. mansoni* (Aronstein *et al.*, 1985a, 1985b, 1986; Rasmussen *et al.*, 1986). Recently, Hillyer *et al.* (1988) reviewed the role of *F. hepatica* cross-reactive antigens in acquired immunity to schistosomes and presented an update of their own efforts to isolate and characterize a 12kD polypeptide of *F. hepatica* that induces 50 to 80% protection in mice challenged with *S. mansoni*.

Monoclonal antibodies developed against *F. hepatica* antigens are reported to protect against challenge with *S. mansoni* cercariae (Hicks and Doughty,, 1987) and soluble tegument antigens of *F. hepatica* can provide protection to mice exposed to lethal doses of *S. mansoni* cercariae (Hillyer and Serrano, 1982).

Other reports describe the development of monoclonal antibodies to further characterize the tegument antigens present in T-1 granules and the glycocalyx of *F. hepatica* (Hanna and Trudgett, 1983; Hanna *et al.*, 1988). Sloan *et al.* (1984) developed monoclonal antibodies to immature liver fluke antigens in order to evaluate their immunoprotective potential. They have isolated an IgM producing cell line which reacted with the surface syncytium and tegument of newly excysted metacercariae, with the tegument and gut of juveniles, and with the gut of adults. Joshua and Bennett (1984) isolated a IgG monoclonal antibody that reacted with surface syncytium of *F. hepatica* and conferred 47 to 55% passive protection.

Immunoglobulins have been detected on the surface of *F. hepatica* parasites harvested from immunocompetent animals and early surface protein labelling studies (Howard *et al.*, 1980). It has been suggested that these immunoglobulins, which have been termed "blocking antibodies", might be converted to more aggressive antibody isotypes by selective vaccination (Mitchell, 1989).

Yan *et al.* (1988) and Feng *et al.* (1985) have attempted to isolate and characterize protective antigens of *Paragonimus skrjabini* using hybridoma technology. They have developed a series of monoclonal antibodies

that recognized specific antigens of *P. skrjabini*.

Recombinant DNA approaches to trematode vaccine development, other than schistosomiasis, are still in their embryonic stage. Zwrita *et al.* (1987) established a cDNA library for *F. hepatica*, and identified and sequenced a female organ-specific protein. Beardsell and Howell (1987a, b) have reproduced *F. hepatica* antigens through co-transformation of established murine cell lines with DNA from *F. hepatica*. This *in vitro* method of producing metazoan antigens allows eukaryotic cells to make post-translational changes which are beyond the capabilities of prokaryotic organisms (Beardsell and Howell, 1987b).

Future advances in the field of vaccine development will depend on a thorough understanding of immune mechanisms and their regulation throughout the course of a parasitic infection. Basic host responses will provide the clues needed to identify which of the many parasite antigens are relevant and biotechnology will provide techniques to isolate and characterize relevant antigens. Hayunga (1989) postulated that one reason "high tech" technologies so far have had little impact on the development of vaccines against trematodes is not that they are inappropriate technologies, but they are examples of technology inappropriately applied. The information database on the immune mechanisms involved and their regulation throughout the course of a trematode infection has been deficient. Knowledge of the basic biology, biochemistry and immunochemistry is essential for the appropriate application of "high tech" technology to vaccine development programs.

Drug Development

Most currently used antihelminthic drugs are products of trial and error approach. Modern biology offers a more rational method for drug design. The combination of biochemistry, molecular biology, protein structure analysis and computer-aided molecular modelling provide the means to elucidate the mode of action of currently used drugs and exploit the differences that exist between the metabolism of the parasite and the host in the design of new generation of antiparasitic drugs (Hart *et al.*, 1989).

Recombinant DNA technology is a quick, powerful and efficient method for dissecting numerous parasite-related peculiarities which are potential targets for drug development. In addition, nuclear magnetic resonance spectroscopy (NMR) is now used in place of more traditional techniques for looking at enzymes and metabolites of parasites. Living parasites can be examined with NMR technology. The presence of 31P (natural phosphorus) and 13C (enriched) nuclei in parasites are determined to investigate carbohydrate metabolism and production of cellular energy compounds in living parasites. Lipids and proteins containing natural phosphorus can also be monitored in living parasites. Matthews *et al.* (1985) demonstrated the possibility of obtaining good quality NMR spectra from intact *F. hepatica* maintained inn NMR tubes. More recently, Matthews and Mansour (1987) reviewed their application of NMR to investigations of glycolytic metabolism and pharmacology of *F. hepatica*. A prime interest of this group is the metabolism of adenylate cyclate, which catalyzes conversion of ATP to cyclic AMP.

Parasite Genetics

When isoenzyme electrophoresis was introduced 20 years ago, it was considered the first major advance in technology for phylogenetic analyses in 50 years. Isoenzyme electrophoresis, however, is appropriate for determining the relationship of organisms that are closely related (Richardson *et al.*, 1986). Recombinant DNA technology is providing insight into the molecular evolution of trematodes and other helminths for this technology is able to directly measure variation. Antigens, isolated and characterized for whatever purpose, are also excellent tools for probing the phylogenetic relationships between helminths (Simpson, 1987). Most modern molecular methods are time-consuming and technically difficult; however, a newly described technique for rapidly obtaining the partial nucleotide sequence of ribosomal RNA is not as time-consuming and technically difficult (Johnson and Baver-stock, 1989). This technique is being applied to investigate phylogenetic relationships among organisms. Liang *et al.* (1986) discussed the importance of this method to helminth phylogeny and demonstrated the usefulness and generality or recombinant RNA sequencing for the systematic phylogenetic classification of parasitic organisms whose tissues are only available in relatively small amounts.

Host Genetics

Scientists, interested in the interruption of disease transmission, have two basic choices. The focus of most efforts has been to modify host resistance or susceptibility through immuno- or chemoprophylaxis or therapy. The other approach is simply to modify the host's genome through introduction genetic characteristics that are considered protective. Traditionally this was done through selective breeding. Molecular genetics and recombinant DNA technology now provide the ability to introduce resistance in an artificial manner (Murrell, 1983). Williams (1987) and Albers and Gray (1987) recently reviewed host genetic resistance and breeding for resistance to parasites and Sher *et al.* (1987) specifically discussed host genetic control of protective immunity to schistosomes. A basic understanding of the molecular basis for resistance coupled with the ability to alter the genome of a species offer the opportunity to modify the host genetically to tip the host/parasite balance in favor of the host in order to reduce or eliminate transmission.

Bioengineering

The durability of trematode egg shells is well recognized. Bioengineers are interested in the molecular structure of trematode egg shells as they represent novel protein biopolymers with potential application in the development of protective paints or biologically based glues. Cordinley (1987) reviewed recombinant DNA studies that have shed light on the formation of schistosome and *Fasciola* egg shell structures. These and other studies suggest that many phylogenetically distant organisms are exploiting cross linking chemistry of certain polypeptides for the construction of protein polymers and several commercial firms are actively trying to duplicate these biological glues and polymers in bacterial systems.

Basic Biology

Although the primary interest in the application of biotechnology to trematode diseases is the potential of these technologies to reduce their associated morbidity and mor-

tality, the more immediate benefit has been the increase in knowledge of host-parasite relationships. Parasites have provided with excellent tools for unraveling the complexities of species interactions at the molecular, cellular, organismal and population levels. Thus, regardless of the practical outcome of the current wave of new technologies on trematode parasitoses, the resulting increase in knowledge of the basic relationships between hosts and their parasites will be of immense value. In turn, improved understanding of host-parasite relationships at the molecular level will continue to expose parasite idiosyncrasies that will make them susceptible to accurate diagnosis, treatment, prevention or control through new biotechnologies.

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QUESTIONS AND ANSWERS :

1. Question: Usually the $^{51}C_5$-release is used to show damage or killing of the labeled cells. The system you used showed the blocking effect of MoAb. Did the effect indicate the specific binding to any receptor and did the $^{51}C_5$-release occur due partly to "shedding phenomena" of the surface membrane ?

   Answer : The data imply the 150.000 MW molecule is involved in the cytopathic/cytotoxic mechanism although further experiments using purified Tf150 need to be done (icy competition experiments) or (direct assay of 150 on targets).

2. Question: What is the crucial nature of the idiosyncracy should be studied in immunological or biotechnological engineering.

   Answer : One should look for specific antigens or metabolic pathways that are unique to the parasite. These antigens or metabolites can then be targeted by antibodies or chemotherapeutic agents.

3. Question: Has there been any biotechnological research which proved that the Schistosome found in Asia and Southeast Asia are all similar species ?
   If no, what will be the appropriate method of biotechnology.

   Answer : Yes, Fletcher et al showed that S.mekongi had a closer evolutionary relationship with S.japonicum compared to S.mansoni. Time of divergence in millions of years calculated from isozyme analysis fits well with similar calculation using non-isozyme techniques.