Multi-author Reviews

Molecular parasitology

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Parasites and molecular biology

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When our Editor, Dr Thomas Seebeck, invited me to write an introduction to this review on Molecular Parasitology, he asked me particularly to comment on the 'old problems' posed to mankind by the eukaryote parasites, before considering how molecular biology might help us to devise new drugs, treatments and prevention strategies. I will attempt to obey his instructions.

Almost all eukaryote phyla contain species which have explored the parasitic way of life, but the species most dangerous to man are either Protozoa or belong to the metazoan phyla which contain the Platyhelminthes (flat worms) or the Nematodes (round worms). These last two are often lumped together as the helminths – a term without proper taxonomic status, but sounding rather more learned than the alternative – parasitic worms!

Many parasitic species infect man: some cause little harm and indeed are often commensal. Others cause chronic diseases which may last for many years whilst a few, such as malaria in the naive subject, can cause acute and rapidly fatal infections. Overall, more than a thousand million of our fellow beings harbour one or more species of parasite. Our livestock are also often subject to parasitic infections, and their death or poor growth leads often to protein deficiencies in the affected areas.

The global importance of parasitic diseases is reflected in the decisions of WHO in its Special Programme on Research and Training in the Tropical Diseases (TDR). Five out of the six diseases set out in the programme are caused by eukaryote parasites: malaria, trypanosomiasis, leishmaniasis, schistosomiasis and filariasis.

Progress in the control of parasitic diseases has been slow compared with some of the successes obtained with viral or bacterial parasites. As discussed below we have at present no successful vaccines against the major parasitic diseases of man, and so control measures currently employed are environmental engineering projects, chemical control of vectors such as mosquitos, tsetse flies and snails, and the chemotherapy of infected individuals. The engineering projects have proved expensive and uncertain in their effects, particularly in countries which are politically or economically unstable. They do, however, often provide additional benefits to the population. Chemical control of both vectors and parasites has often shown considerable promise. Combined use of insecticides and antimalarial drugs, together with public health education, almost eradicated malaria from the Indian sub-continent in the 1950s, but failure to *maintain* control measures, and the development of resistance to chemical agents by both parasites and mosquitos, led to its resurgence over wide areas.

Vaccination, so successful in respect of some viral and bacterial diseases, has yet to make a significant impact against the parasitic diseases of man. We have a handful of vaccines in use against the parasites of domestic animals, using attenuated parasites or involving deliberate infection and drug treatment, but we have no anti-parasite vaccine against human disease which has been used in more than a handful of volunteers. There are a number of reasons for this unhappy state of affairs. Certainly, lack of funding has been a major problem in the past, but funding by national governments, WHO, EEC and private organizations such as the Wellcome Trust, the Rockefeller and the Edna McDonnel Clark Foundations, have all provided additional funds in recent years. We are now in a position to identify at least the two major problems that still hinder progress towards effective vaccination against parasites. The first relates to the difficulties in cultivating parasites in vitro. We can successfully grow many, if not all, viruses and bacteria in vitro, and then attenuate or kill them to obtain vaccines, but this has not yet proved possible with the eukaryote parasites. Secondly, and probably more importantly, we have discovered that parasites have evolved various strategies for evading host immunity. These strategies, involving antigenic diversity, antigenic mimicry and disguise, and the induction of a state of 'immune confusion' in the host, make the development of effective anti-parasite vaccines difficult – and in some cases, probably impossible.

I published my first scientific paper on the biology of parasites in 1956 [29], which was just three years after Watson and Crick [33] had published their ideas on the basic structure of DNA. But there was a year or so to go before the semi-conservative replication of DNA was unequivocally demonstrated and several more before the work of Nirenberg [17], Khorana [10] and their collaborators elucidated the genetic code. All studies at this time were, of

course, restricted to prokaryote organisms, and mainly to a particular type of *Escherichia coli*. We assumed, however, that what turned out to be true for *E. coli* was going to be true for all living things, and, with certain very important provisos, our assumptions have been correct. But up until about 1980, what we now call molecular biology added very little to our knowledge of eukaryote parasites. We did learn a great deal about parasites over this period but our improved understanding was brought about by advances in three areas: 1) improved knowledge of the anatomical structure of parasites by the development of transmission and, later, scanning electron microscopy; 2) advances in basic biochemistry and biochemical techniques which made us much better informed about the metabolic activities of parasites; and 3) advances in immunology which not only enlightened us greatly on the details of host-parasite relationships, but provided us with highly specific tools in the form of antibodies, particularly monoclonal antibodies, for analysing the molecular architecture of parasites.

Associated with these advances were much-improved opportunities for intercourse amongst parasitologists – through meetings and publications. For example, my own personal recollections include attendance at the first meeting of the British Society of Parasitology at Cambridge in 1962, the first International Congress of Parasitology in Rome in 1964, and the first European Multicolloquium of Parasitology in Rennes in 1971. And besides the old established journals such as *Parasitology*, the *Journal of Parasitology* and the *Journal of Tropical Medicine*, we produced a whole host of new ones, some catering to specialised aspects of the subject; *Experimental Parasitology*, *International Journal of Parasitology*, *Biochemical and Molecular Parasitology*, *Parasite Immunology* and *Parasitology Today* all come to mind.

Technical advances, better communications and improved research funding inevitably led not only to a better understanding of how parasites worked but allowed us to define problems in such a way that they could be tackled by the molecular biologists as they developed their own techniques and skills. I would like to illustrate this by discussing briefly aspects of the biology of two parasites in which I have long been interested – African trypanosomes and schistosomes.

Since the turn of the century we had been aware that African trypanosome infections followed a cyclical pattern, but it was not until 1965 that the work of Gray[6] in West Africa allowed us to appreciate the true nature of antigenic variation in these organisms. Somewhat later, Vickerman and Luckins[32] visualized the variant antigens in the surface coat of the parasite by transmission electron microscopy, and first Le Page[11] and then Cross[4] carried out detailed biochemical studies on these variant specific glycoproteins (VSG). Thus a firm basis had been laid by the late seventies and early eighties for the research of molecular biologists[1,27], who have largely elucidated the genetic basis for the astonishing degree of antigenic variation seen in these parasites. Unfortunately, the immune evasion strategy evolved by this parasite would seem to be able to defeat all attempts to develop effective vaccines against African trypanosomes. Research in the area might yet, however, lead to alternative methods of control. For example, if we understood fully the mechanisms whereby the trypanosome switches from one VSG gene to another, and could block this by chemical means, we would have a most effective chemotherapeutic agent. Trypanosomes that were prevented from VSG gene switching would be easy victims for specific antibody-mediated immunity.

Turning to schistosomes, it was recognized in the early 1960s that schistosome infections in man could be extremely long-lived, and that a single infection in a rhesus monkey might last for more than a year even though the worms were eventually killed. Smithers and I[23] showed, however, that although an infected rhesus might be unable to rid itself of an established infection if could often resist a second challenge infection. We later[24] showed that the actual transfer of adult parasites into the mesenteric veins of a rhesus would immunize the monkey against a challenge by cercariae, but again the transferred *adult* worms would survive. This somewhat paradoxical situation where the presence of the adult worms stimulated an effective immune response against cercariae, but were themselves unaffected, we termed 'concomitant immunity'[25]. Eventually we came up with the idea that although the adults could act as immunogens they were possibly disguising themselves through the acquisition of molecules of host origin, or 'host antigens' as we termed them. We were able to demonstrate that this was indeed the case, and to show, with the aid of Hockley's electron microscopy skills, that the host antigens were located where they should be – at the surface of the parasite[26]. Together with Clegg and other workers[3,5] we were able to show that schistosomes grown in human blood would develop the appropriate blood group specificity, probably incorporating these molecules as glycolipids into their surface membranes.

Again the electron microscope had an important part to play as McLaren and Hockley[7,13] showed that blood-dwelling trematodes, but not trematodes living elsewhere in their hosts, possessed a peculiar and characteristic *double* outer membrane which may be specialized for the adsorption of glycolipids of host origin.

Schistosomes may have additional defences against immune attack such as increased surface membrane turnover[19]; immunoglobulin Fc receptors[8,30]; and the ability to stimulate 'blocking antibodies'[9,34] which may hinder the binding of more effective antibodies. Additionally, the 'lungworm' stage of the parasite appears to possess an as yet unknown defence against antibody-mediated cell cytotoxicity, which certainly merits further study[14].

It is clear, therefore, that the research aimed at producing vaccines against schistosomes, so ably reviewed in

this volume[28], will also have to take into account the defences of the parasite. It may be that the schistosome with its ability to acquire an antigenic disguise presents only a fleeting target to the immune system, and that a very powerful immune response will be required for its destruction. We may also speculate that if we could devise some chemotherapeutic strategy which would hinder the uptake of host antigen, that the wolf *minus* its sheep's clothing would be rapidly recognised and destroyed.

Although my first paper on parasites was concerned with immunity to malaria, I have been only a spectator of research in this area for many years. It does, however, seem to me to be the most promising major human parasitic disease for immune intervention to be effective. Although *Plasmodium* shows a degree of antigenic diversity, including stage-specific antigens, this is much less than that seen in the African trypanosomes. There is no evidence for significant acquisition of host antigens, although the intracellular location of the parasite gives at least some degree of protection from humoral immunity. Epidemiological evidence also supports the idea that immunity to malaria can and does develop in endemic areas.

Some early success with the culture of blood stages of *P. falciparum* gave rise to hopes that large amounts of malaria antigens might become available as material for vaccines[31]. Unexpected problems in scaling up production now make the feasibility of this approach questionable, and it looks as though our only chance lies in the successful cloning of genes coding for key antigens, and their expression in suitable vectors. Certa's article[2] in this volume reviews these advances and the associated problems fully, and so I need just express a few general thoughts.

There is a very great need – but no ready market – for anti-malarial vaccines. Those who need them cannot afford them and so considerable altruism will be required from the people of the developed world in order to provide help for the victims of the developing world. For example, we will certainly require the services of the genetic engineering and pharmaceutical firms in order to produce anti-malarial vaccines – but who is going to pay for them? Certainly not those who need them most – the children of the endemic malaria regions of the developing world!

We must then rely on the Donor Agencies of the developed world for support, and here again we have a problem in steering a middle course between the twin hazards of excessive optimism and profound pessimism. It is my belief that we have erred somewhat on the side of optimism with regard to anti-malarial vaccines. Over the last 20 years I seem to have heard, at very regular intervals 'A malarial vaccine in 5 years!', or 'Malaria vaccines? Just round the corner, Old Boy!'

I believe that we have rather underestimated our opponents. Workers in the field – particularly those coming from disciplines other than parasitology – have looked upon eukaryote parasites as being rather larger and somewhat more complex prokaryote parasites. But protozoa and helminths are eukaryotes with a true nucleus, with genes containing introns and exons, and with the ability to 'tailor' mRNS before it passes to the ribosomes. Operating on the very imperfect criteria of genome size, protozoa are about ten times, and helminths a hundred times more complicated than bacteria[22]. This complexity has, I believe, enabled eukaryote parasites to evolve the sophisticated defences against immune attack which enabled me to sum up a symposium on parasite evasion mechanisms with a paper entitled 'Parasites as immunologists!'

If I were about to enter the field of genetically engineered vaccines against eukaryote parasites I might be tempted to investigate such vaccines against parasites of livestock. There could be a number of reasons for this decision. First, almost all of the anti-parasite vaccines currently in use are aimed at preventing disease in animals: vaccines against protozoan infections of cattle, *Babesia* and *Theileria*[16], and against the nematode lungworm of cattle[20]. Secondly, it is permissible to take greater risks of vaccine-induced pathology with young animals than with young children; failures with animals can often be eaten! Thirdly, infections with eukaryote parasites occur in animals in developed, as well as in developing countries, and so we might enjoy a financial return on investment in research. I have a feeling that the euphoria which attended the early attempts to vaccinate against human parasites – especially malaria – has somewhat evaporated. Thus a significant success against an important parasite of livestock would restore confidence in our long-term aim of vaccinating against the major parasites of man.

To help readers who are not primarily parasitologists, I would like to draw to their attention three reviews which report the Autumn Symposia of the British Society of Parasitology. They are all published in *Parasitology* and are as follows: 'Parasite evasion of the immune response' (Parkhouse, 1984)[18]; 'Parasites and molecular biology: applications of new techniques' (Simpson, 1986)[21]; and 'Vaccines and vaccination strategies' (McLaren 1989)[12]. Together with the articles in this volume they will confer competence upon the reader.

Finally, I am aware that I have been highly selective in the research I have described and the literature I have quoted. But I was asked to provide an introduction to the field and not a comprehensive review. I trust that my friends and colleagues whose valuable research I have not mentioned, will forgive me.

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mRNA processing in the Trypanosomatidae

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Summary. Members of the Trypanosomatidae, which include the African trypanosomes, the American trypanosomes and the leishmanias, cause disease in vast proportions in man and his livestock and are a major detrimental factor to the social and economic well-being of the third world. Current research using the techniques of molecular biology has revealed two unusual types of mRNA processing in these protozoans; these are the addition of a shared leader sequence to the 5′ ends of nuclear mRNAs by a mechanism of *trans* splicing, and the insertion and deletion of specific uridine residues in mitochondrial transcripts by RNA editing. The presence of these two mRNA processing pathways in the Trypanosomatidae has profound consequences for the organization and expression of their genetic information.

Key words. Trans splicing; spliced leader addition; RNA editing.