# MOLECULAR PARADIGMS FOR ERADICATING HELMINTHIC PARASITES

Austin MacInnis, Organizer
January 24 - January 31, 1987

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# *Molecular Paradigms in Development*

ACTIN AND COLLAGEN GENES IN C. ELEGANS

**C 001** David Hirsh, Synergen, Inc., 1885 33rd St., Boulder, CO 80301

The nematode C. elegans contains four actin genes. Three of the genes are clustered within a 12 kb segment of DNA. Mutational analysis and in situ hybridization studies of the actin mRNAs indicate that the three clustered genes are predominantly transcribed in muscle cells and the fourth gene encodes cytoplasmic actin. The genes display very little transcriptional modulation during post-embryonic development. Analysis of the 5'-ends of the mRNAs from the three clustered genes reveals sequences that cannot be found anywhere in or near the genes and therefore must arise from elsewhere.

In contrast to the actin genes, the collagen genes are numerous--40 to 150 genes per genome, and they are differentially transcribed during post-embryonic development. At each molt, an added set of genes is activated to synthesize collagens that comprise the cuticle.

Gene fusions have been constructed of actin and collagen promoters with bacterial genes that act as reporters of promoter activation in order to measure developmental regulation of the individual members of these two gene families.

**C 002** ASEXUAL AND SEXUAL DIFFERENTIATION IN CESTODES, J.D.Smyth, Dept. of Medical Helminthology, London School of Hygiene & Tropical Medicine, Keppel Street, (Gower St.), London WC1E 7HT, England.

The ability to undergo asexual reproduction during various stages of their life cycles is common in the Protozoa and certain invertebrate Phyla. In the Cestoda, asexual reproduction is largely, but not exclusively, confined to the Cyclophyllidea, occurring especially in the families Mesocestoididae, Hymenolepidae and Taeniidae. The development of reliable techniques for the in vitro cultivation of the Echinococcus granulosus[1,2,3] and E. multilocularis (Taeniidae) - the causative organisms of hydatid disease - and Mesocestoides corti[4,5,6] (Mesocestoididae) have enabled these phenomena to be investigated in detail. It is now possible to manipulate these species in vitro so that they develop either sexually (resulting in the development of a strobila) or asexually (resulting in the development of hydatid cysts (Echinococcus) or tetrathyridia (Mesocestoides).

In E. granulosus, an important factor which triggers strobilar development appears to be the presence of an appropriate solid protein substrate with which the scolex can make contact[1]. In contrast, the related species, E. multilocularis, can differentiate sexually in a monophasic medium[3]. In both species, somatic and genital differentiation appear to be only loosely linked and, under certain conditions, the former is inhibited and remarkable 'monozoic' forms develop[7,8]. In M. corti, the major factor inducing sexual differentiation in vitro appears to be the maintenance of anaerobic conditions and a pH>7.4 [9]. Almost nothing is known regarding the molecular basis for asexual and sexual differentiation in these species but is is possible to build up a simplistic model to account for these phenomena[9]. Since Echinococcus is a self-fertilizing hermaphrodite, the occurrence of asexual reproduction has important implications for the development of new 'strains' of these species and the importance of this phenomenon in the epidemiology of hydatid disease is discussed [10].

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**C 003** NEMATODE DEVELOPMENT AND THE PROBLEM OF GERM LINE-SOMA IDENTITY, Heinz Tobler, Fritz Müller and Pierre Aeby, University of Fribourg, 1700, Fribourg, Switzerland.

The ontogenetic development of nematodes is characterized by an early restriction of the developmental potentialities of individual blastomeres. This so-called "mosaic" type of development can be demonstrated by experimental embryological techniques, e.g. by setting specific lesions in early cleavage stages and following the respective defects in the differentiated organisms. The cell lineage of the embryo of the horse parasite Parascaris equorum has been established already by T. Boveri at the beginning of this century by careful cytological analysis. Using more sophisticated methodology, the entire developmental cell lineage from the zygote to the adult organism of the free living nematode Caenorhabditis elegans has now been worked out. Nematode development is furthermore characterized by the occurrence of cell constancy, programmed cell death, the absence of any regulation or regeneration phenomena, and by the early segregation of germ line and somatic cell lineages. There is no evidence for the totipotency of somatic cell nuclei, and consequently no a priori necessity for genetic identity of germ line and somatic cells. The process of chromatin diminution which was discovered one hundred years ago in P. equorum actually demonstrates that presumptive germ line and somatic cells differ in their content of chromatin. At least 10 more nematode species undergo chromatin diminution, whereas there is no proof that this process takes place in C. elegans.

Molecular analysis of the chromatin diminution process in the hog parasite Ascaris lumbricoides carried out in our laboratory shows that about one fourth of the total amount of germ line DNA is expelled from the presumptive somatic cell lineage during chromatin diminution. Over 99%, but not all of the satellite DNA sequences present in the germ line genome are removed from the presumptive somatic cells. Genes coding for rRNA and tRNA are not eliminated. On the other hand, single copy DNA sequences are clearly expelled from the presumptive somatic cell lineage, thus demonstrating that in A. lumbricoides the germ line and somatic cell lineages are qualitatively different. We have isolated a transposon-like element (Tas) which is present in about 50 copies per haploid germ line genome and exists in at least 2 different molecular forms. Whereas only about one fourth of the Tas-1 elements are expelled from the somatic cell lineage, all Tas-2 copies are specifically eliminated and are thus confined to the germ line cell lineage. Their genomic organization, transcriptional activity and the attractive idea of their possible involvement in the chromatin diminution process are currently being investigated in our laboratory.

## *Receptors, Transducers and Hormones*

**C 004** RECEPTORS, TRANSDUCERS AND HORMONES IN PARASITIC WORMS, Tag E. Mansour, Joan M. Mansour and Max H. Iltzsch, Dept. of Pharmacology, Stanford, CA 94305.
Serotonin (5-HT) has been implicated in the regulation of neuromuscular activity and of glycolysis in several trematodes (1). We are studying 5-HT receptors in these parasites and the molecular entities involved in their signalling system. Serotonin receptors in F. hepatica and S. mansoni are linked to a very active adenylate cyclase. Studies on the effect of a series of 5-HT antagonists on activation of adenylate cyclase and on binding of radiolabeled ligands showed that the fluke receptors are different from those that have been studied in mammals (2). Cyclic AMP acts as the second messenger in the signalling system from the serotonin receptors to the effector enzymes. GTP, which binds to a signal transducer protein (G-protein), is necessary for full transmission of the signal from the receptor to adenylate cyclase. Guanosine 5'-(3-O-thio)triphosphate (GTPγS) and Gpp(NH)p, both poorly hydrolyzable GTP analogs, activate adenylate cyclase by binding to G-protein. Using bacterial toxins and photoaffinity labeling with [$^{32}$P]-8-$N_3$GTP, we have identified a family of G-proteins in F. hepatica (3). These include a 53 kDa protein and a 45 kDa protein which are similar to the forms of the α-subunit of the stimulatory G-protein in mammals ($G_s$). A 43 kDa protein, equivalent to the inhibitory G-protein ($G_i$) in mammals, may be part of the transmembrane signalling system in the liver fluke. Several other G-proteins have been identified but their functions are still uncharacterized. Binding of 5-HT to the receptors leads to conversion of $G_s$ to the active conformation and subsequently to activation of adenylate cyclase. The increase in the level of cyclic AMP results in activation of a protein kinase which phosphorylates rate-limiting enzymes in glycolysis (e.g., phosphofructokinase). Three cAMP-dependent protein kinases have been partially purified from F. hepatica and have been designated $A_1$, $A_2$, and B. The enzymes differed with respect to their molecular weights and activation by cAMP. Physical and kinetic properties of the catalytic subunit of these enzymes were similar to those reported for the mammalian enzyme. Regulatory subunits from the parasite or from mammals can inhibit catalytic subunits from either source, indicating that salient features of the enzyme have been conserved in evolution. The appearance of the 5-HT/cyclic AMP signalling system is one of the events that occur in the first 48-96 hrs following the development of the parasite from a free-living cercaria to a schistosomule (4). The implication is that this signalling system may be closely linked to maturation of the schistosomule into an adult parasite. This fact, added to the extraordinarily high activity of this system in the parasite, may suggest growth-related functions in addition to controling glycolysis. (Supported by USPHS Grant AI 16501).

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**C 005** MOLECULAR HYBRIDIZATION AND IMMUNOLOGICAL DATA SUPPORT THE HYPOTHESIS THAT THE TAPEWORM SPIROMETRA MANSONOIDES WAS TRANSFECTED WITH A HUMAN GROWTH HORMONE GENE. C. K. Phares and G. S. Cox, Dept. of Biochem., Univ. of NE Med. Ctr. Omaha, NE 68105.
Plerocercoid growth factor (PGF), a substance produced by the plerocercoid stage (metacestode) of the tapeworm Spirometra mansonoides, is associated with accelerated growth of the host leading to the hypothesis that plerocercoids produce a "GH-like" factor. This has been substantiated, and PGF is more similar to human growth hormone (hGH) than it is to other growth hormones. Among the characteristics which distinguish hGH from other mammalian GHs include: hGH has both somatogenic and lactogenic activities, hGH has unique receptors in female rabbit liver, and only hGH binds to receptors in human tissues (no other GHs are active in humans). However, there are biological actions shared by all GHs. Besides growth promotion, all GHs have acute, insulin-like activities in GH-deficient animals and all GHs have lipolytic/anti-insulin/diabetogenic activities. Biological similarities between PGF and hGH include: expression of both somatogenic and lactogenic activities, displacement of hGH from its receptors in rabbit liver and human cells. Of importance are differences in biological activities. PGF stimulates persistent (vs transient for hGH) insulin-like effects in normal animals (vs GH-deficient for hGH) and PGF does not possess lipolytic/anti-insulin/diabetogenic activities. Murine monoclonal antibodies (Mabs) have been used in studies to analyze the structure/function relationship of hGH and its close relatives. We examined the cross-reactivity of PGF with anti-hGH and anti-human placental lactogen (hPL) Mabs. The specificities of the MAbs varied from very strict binding to hGH (QA68) to almost equal binding to hGH and hPL (EB1, and EB2 [anti-hPL]). PGF cross-reacted with both anti-hGH and anti-hPL Mabs but with varying affinities. The immunopotency of PGF for the strictly hGH-specific QA68 was 68% that of hGH. PGF was recognized by anti-hPL MAbs with immunopotencies equal to 55% (EB2) and 29% (EB1) of hGH. Closely related structural genes from the same or different species can be identified by conducting molecular hybridization experiments between cDNA and DNA (or RNA). We conducted such experiments under stringent conditions with $^{32}$P-labeled hGH cDNA and plerocercoid DNA and RNA. Autoradiographs of both Southern and Northern blots suggest that sequences in plerocercoid DNA and RNA have significant homology to sequences in the hGH cDNA. Therefore, biological, immunological and molecular hybridization data provide evidence that PGF is very similar but not identical to hGH. During the association of the plerocercoid stage of S. mansonoides with its human host a hGH gene may have been virally transferred into plerocercoids. During the evolution of PGF growth promotion and insulin-like actions have been retained but the anti-insulin/diabetogenic activities were lost. Supported by NIH AM37030.

**C 006** SCHISTOSOMA MANSONI: APICAL MEMBRANE/ENVELOPE SYNTHESIS, SIGNAL TRANSDUCTION, PHOSPHOPROTEINS AND MOLECULAR CLONING, R. Podesta, S. Karcz, M. Ansell and E. Silva, University of Western Ontario, London, Canada N6A 5B7.

Similar to other parasitic flatworms, the basic functional unit at the surface of S. mansoni is a syncytial epithelium but differs in that the syncytium is covered by an envelope (E). As in syncytial tissues of other organisms, the syncytial epithelium of S. mansoni has two major functions - transepithelial transport and protection from the host's immune response. We have suggested previously that the latter is the major adaptive feature of syncytial tissues, which are efficiently designed for rapid synthesis and turnover of the apical membrane in a typical stimulus-secretion coupling mechanism. Serotonin (5-HT) stimulated synthesis of the apical plasma membrane (APM) while A23187 and $C_{3b}$ stimulated E synthesis. $C_{3b}$ but not 5HT accelerated $Ca^{++}$ influx. 9-10 polypeptides associated with a fraction containing the APM were phosphorylated under control conditions. E polypeptides were not phosphorylated. The effects of reaction time, ATP and cation concentrations on phosphorylations were examined. On Western blots, a 24 Kd polypeptide was labelled with $\gamma$-$^{32}$P-ATP but not $\beta$-$^{32}$P-ATP. Similar results were obtained with a 22 Kd polypeptide labelled with $\gamma/\alpha$-$^{32}$P-GTP. 5-HT stimulated phosphorylation of all APM phosphoproteins while $C_{3b}$ inhibited phosphorylation of these polypeptides. Using an anti-APM serum and epitope selection, a cDNA clone for a 24 Kd phosphoprotein has been isolated. This APM polypeptide separates into a Triton X114 phase and binds to a variety of lectins. A model of signal transduction leading to APM/E synthesis will be presented based on these data.

**C 007** PROTEIN KINASES IN NEMATODES, Rolf Walter, Bernhard-Nocht-Institut, Abt. Biochemie, D-2000 Hamburg 4, F.R. Germany

Cyclic nucleotide, calcium-calmodulin and calcium-phospholipid dependent protein kinases are integrated in current concepts of cellular regulation and signal transduction. The occurrence of at least one cyclic nucleotide dependent protein kinase, properties of which are characteristic of type I cAMP protein kinase, has been well-established for various helminths (1-7); phosphofructokinase from Ascaris suum and Fasciola hepatica has been identified as one of its physiological substrates (8,9). Recently, calcium-phospholipid dependent protein kinase, which is expected to have a crucial role in signal transduction and transmembrane control of cellular functions, has been reported from filarial worms (10). In addition, forthcoming studies on Onchocerca volvulus indicate a protein kinase, the activity of which depends on calcium/calmodulin and which might be identical with phosphorylase kinase. The occurrence of second messenger independent protein kinases has been reported from nematodes, too. Beside a pyruvate dehydrogenase kinase (11), two casein/phosvitin kinases were isolated and characterized from O. volvulus, Ascaridia galli and Nippostrongylus brasiliensis (2,5,7), which differ with respect to molecular weight, sensitivity for heparin and suramin. Marked variations in the phosvitin-phosphorylating activity during development of filarial worms -as shown for adults, microfilariae and infective larvae- indicate the involvement of protein kinases in developmental processes (12).

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# *Molecular and Cellular Neuroparasitology*

**C 008** CHOLINERGIC-INDUCED CHANGES IN MECHANISMS INVOLVED WITH INTRA CELLULAR $CA^{++}$ CONCENTRATION OF S. MANSONI, J. Bennett, D. Semeyn and R. Pax, Department of Zoology and Pharmacology, Michigan State University, East Lansing, Michigan 48823. To date most of the work concerning the neurobiology of schistosomes has focused on the action of neuropharmacological agents or putative neurotransmitters on parasite muscle activity or biochemical processes. Thus, many publications have appeared describing the action of neurotropic drug's on schistosome muscle activity but to date we have no understanding, with the possible exception of serotonin, about the molecular mechanisms which mediate the action of these drugs.

Acetylcholine appears to be a major neurotransmitter in schistosomes yet our knowledge about its action on the parasite's muscle is descriptive, since all we know is that it causes a flaccid paralysis. Our research has been focused on trying to understand the mechanism which explains the action of this neurotransmitter on the parasite. Our research indicates that the action of cholinergic agonists is energy dependent but is not altered by changes in the concentration of the major inorganic components surrounding the parasite. Calcium selective ionophores will reverse the paralytic action of cholinergics and reinitiate spontaneous contractions. When the parasite's musculature is contracted and depolarized by high concentrations of potassium, cholinergics will still relax the musculature but have no effect on the polarity (membrane potential) of the depolarized muscle cells.

The above results support the idea that cholinergic agonists may alter the tonic state of the parasite's muscle cells by lowering the concentration of calcium within these cells through voltage independent gates, i.e., receptor operated channel. Further proof of this concept was obtained when we demonstrated that cholinegic agonist stimulated the efflux (but not influx) of $Ca^{++}$ from the parasite and that this stimulation was correlated with an activation of the parasite's $Ca^{++}$ ATPase by cholinergic agonist. These results provide evidence that the cholinergic induced effects (flaccid paralysis) on schistosome muscle is mediated by a receptor operated, voltage independent, activation of a membrane bound $Ca^{++}$ ATPase.

**C 009** PLATYHELMINTH NEUROTRANSMITTERS, D.F. Mettrick, Department of Zoology, University of Toronto, Toronto, CANADA M5S 1A1.

The success of any molecular arsenal aimed at the eradication of helminth parasites will ultimately depend on the precision with which the target can be characterized. Although several putative neurotransmitters have been described from platyhelminths, the evidence has been based on indirect methods or on histochemical data. Progress has been hampered by the inability to isolate platyhelminth nervous tissue from mesenchyme. Our approach has been holistic, and on the basis of anatomical, biochemical, histological and physiological evidence we can identify two candidates as platyhelminth neurotransmitters. The major inhibitory neurotransmitter is acetylcholine (ACh). ACh inhibits the contractions of whole and isolated muscle strips of *Hymenolepis diminuta*, *H. microstoma* (Cestoda) and *Fasciola hepatica* (Trematoda). ACh release is tonic and the effects of this neurotransmitter on muscle activity are mediated by receptors on the muscle membranes. Choline, ACh, and the enzymes that synthesize and degrade ACh, choline acetyltransferase and acetylcholinesterase, have been biochemically identified and measured in homogenates of worm tissue. Histochemical techniques at the ultrastructural level have been used to localize the presence of acetylcholinesterase within the synaptic clefts of neuro-neuronal contacts in the cerebral ganglia of *F. hepatica*. The pharmacological characterization of the ACh receptors reveal receptors that are neither purely nicotinic nor muscarinic in nature and probably reflects the primitive phylogenetic position of the flatworms. Similar studies to characterize the neurotransmitter that is stimulatory in these animals have not been as successful. Most investigators consider 5-HT as the prime candidate for this role but our studies show that glutamate produces a more consistent stimulation of muscular activity in cestodes; the specific receptors of this amine have been pharmacologically identified in cestodes. There are numerous other potential neurotransmitters, including dopamine, noradrenaline and several neuropeptides that have been reported from cestodes and trematodes but which have not been investigated. A complex picture is emerging as the methods of investigating these difficult preparations are refined. The possibility of the development of a viable weapon, directed at the nervous system of platyhelminths, is presently in its infancy.

C 010 NEUROTRANSMITTER ENZYMES AND DRUG RESISTANCE IN *C. elegans*, Richard L. Russell, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

We have previously described four genes concerning neurotransmitter enzymes in *C. elegans*. Three of these, *ace-1*, *ace-2*, and *ace-3*, are apparent structural genes, respectively, for three separable and kinetically distinct classes, A, B, and C, of acetylcholinesterase (AChE), the degradative enzyme for the excitatory transmitter acetylcholine (ACh). This three-gene family appears internally redundant; elimination of any single AChE class, by mutation of the appropriate gene, has no behavioral or developmental effect. However,the family as a whole appears essential; elimination of all three AChE classes, in the *ace-1; ace-2; ace-3* triple mutant, produces marked paralysis and failure to develop after hatching.

The fourth gene we have identified, *cha-1*, is the structural gene for choline acetyltransferase (ChAT), the biosynthetic enzyme for ACh. Extreme mutations in this gene produce lethality, but slightly less severe ones, in which only 1-2% of the normal ChAT levels survive, produce a characteristic syndrome of uncoordination, slow growth, small adult size, and, interestingly, resistance to inhibitors of AChE. All features of this syndrome are recessive; *cha-1* / + heterozygotes display none of them. The resistance to AChE inhibitors, which may have practical importance, appears directly due to the reduced synthesis of ACh; this should lead to reduced ACh release at synapses, and therefore reduced ACh accumulation in synaptic clefts (and less drastic postsynaptic results) when AChE is inhibited. Thus in the presence of inhibitor, the mutations appear to restore partially an original balance, by reducing levels of ACh release to compensate for inhibitor-reduced levels of degradation.

*C. elegans* mutants resistant to AChE inhibitors can be induced at appreciable frequency, and many have been isolated. Only about 3% fall in *cha-1*, but the remainder fall almost entirely in about a dozen other genes. In almost all cases such mutants display the same syndrome as with *cha-1* mutants, and all features of the syndrome are again recessive. I suggest that each of these genes is concerned with some step in the packaging and release of ACh, that each mutant is defective in the corresponding step and therefore in overall ACh release, and that each mutant's syndromic features (including its inhibitor resistance) are based on this common property. In this view, the relatively high frequency of resistant mutants can be understood because several genes can mutate appropriately, and in each the kind of mutation producing resistance is the most common type, i.e. one which reduces gene function. By extension, for any transmitter which has an active removal mechanism (e.g. reuptake or metabolism) pitted against release, drugs targeted either at release or removal can be expected to have resistant mutants arising at relatively high frequency, by mutational reduction of the opposing process to restore a partial balance.

C 011 NEMATODE NEUROBIOLOGY USING ASCARIS AS A MODEL SYSTEM, Antony O.W. Stretton, Ralph E. Davis, James D. Angstadt, Judith E. Donmoyer, Carl D. Johnson, and Jeffrey A. Meade, University of Wisconsin Madison,WI 53706.

The motor nervous system of Ascaris contains about 90 motorneurons and interneurons. In order to understand its function, we have carried out an anatomical analysis of the wiring diagram. Individual neurons are recognizable by their morphology, their patterns of synaptic connectivity to other neurons and to muscle cells, and in many cases, by the neurotransmitter that they contain or synthesize. The system can also be investigated by electrophysiological techniques, and the electrical properties of the cells and their synaptic connections have also been investigated. Taken together, the anatomical and physiological results suggest a model for nematode locomotion. The model is based on the cellular properties of inhibitory motorneurons and their synaptic contacts with excitors. The inhibitors receive their input from excitors such that ventral inhibitors are controlled by dorsal excitors, and dorsal inhibitors by ventral excitors, thus mediating reciprocity between dorsal and ventral muscle fields. When inhibitors are depolarized, they oscillate, and synaptic connections keep dorsal and ventral inhibitors out of phase. However, the oscillations are too fast to account for locomotory movements. We are currently examining the mechanisms for generating the appropriate slow signals.

## *Cloning Antigens and Other Genes*

**C 012** CLONING AND EXPRESSION OF SCHISTOSOMA JAPONICUM DNA, Bernard J. Scallon and Clint E. Carter, Vanderbilt University, Nashville, TN 37325. A library of randomly sheared *Schistosoma japonicum* genomic DNA fragments was constructed in the bacteriophage expression vector, λgt11. A portion of the library was screened with sera pooled from 100 human schistosomiasis patients from China. Another portion was screened with sera collected from rabbits eight weeks after they were infected with 1000 cercariae. Eleven DNA clones whose recombinant gene product reacted with the human sera and four that reacted with the rabbit sera were purified and introduced into *E. coli* to make lysogens. The lysogens were induced to make their fusion proteins and their lysates characterized by Western blots using various antibody preparations. The fusion protein of one clone reactive with the human sera was also reactive with 7 week infected mouse sera. Another was reactive with 16 week infected mouse sera. A clone isolated with the infected rabbit sera has been shown to encode part of a 70 kilodalton antigen that is present in the nervous tissue of male and female adult worms, the dorsal tegument of male adult worms, and the vitelline membrane in eggs. The fusion proteins in these and other clones of interest are being purified and tested for their potential use in immunodiagnosis and vaccine development.

**C 013** ANTIGEN EXPRESSION IN BRUGIA LYMPHATIC FILARIAE. Murray Selkirk, Paddy Rutherford, Felix Partono* and Rick Maizels. Department of Pure & Applied Biology, Imperial College of Science & Technology, Prince Consort Road, London, UK. *Department of Parasitology, Faculty of Medicine, University of Indonesia, Salemba Raya 6, Jakarta, Indonesia. Lymphatic filariasis is a parasitic disease which can take many forms ranging from asymptomatic infection to chronic obstructive lesions. Immunochemical analysis has been applied to a restricted set of molecules from filaria of the genus Brugia, with particular attention paid to highly antigenic components of the cuticle. Approximately one hundred immunoreactive clones have been isolated from a cDNA expression library. Differential screening with sera from naturally infected Indonesians of variant parasitological status has revealed a small number of antigenic peptides which are recognised exclusively by amicrofilaraemic individuals. Purified recombinant proteins (fused to β galactosidase) have been used in ELISA to determine antibody levels in people with different clinical symptoms. One of the most immunodominant proteins identified to date is closely homologous (70% homology at the amino acid level) to heat shock protein 70 from Drosophila. This protein is expressed in all stages examined (adults, microfilaria and third stage larvae), although synthesis at high levels appears to occur exclusively in stages resident in the vertebrate host. An increased rate of synthesis of hsp 70 can be induced in third stage larvae by temperature shift (from 26°C to 37°C), and culture at 45°C induces a classic heat-shock response, with repressed synthesis of all but a restricted set of proteins. Antibody activity appears to be directed predominantly to a C-terminal tetrapeptide repeat unit also found in the analagous protein from Plasmodium falciparum. (Bianco et al, in press)

Bianco AE et al (1986) A repetitive antigen of Plasmodium falciparum that is homologous to heat shock protein 70 of Drosophila melanogaster. Proc. Natl. Acad. Sci. (in press)

**C 014** THE UTILIZATION OF CLONED GENES IN THE STUDY OF SCHISTOSOME SPECIATION DEVELOPMENT AND IMMUNITY.

Andrew J.G. Simpson, David Rollinson, Vanderlei Rodriguez, Charles Kelly, Matty Knight, Tina Walker, Fiona Hackett, Maliha Chaudhri, S. Ronald Smithers; Division of Parasitology, National Institute for Medical Research, Mill Hill, London, NW7 1AA.

Cloned genes permit direct analysis of the schistosome genome and its expression as well as the production of polypeptides relevant to the study of infection. Thus cloned genomic DNA fragments containing repetitive elements have been used in the study of intra-specific variation and schistosome phylogeny. In particular, the gene encoding the ribosomal RNA has been studied which exhibits diversity in the non-coding regions but which is highly conserved in the coding regions. Clones encoding a highly expressed, developmentally regulated gene linked with schistosome egg production have been used to investigate the characteristics of schistosome maturation and oogenesis. cDNA clones containing the homologous gene from both S. mansoni and S. haematobium, which encodes a glycine-rich polypeptide thought to be an egg shell protein, have been obtained and sequenced. In addition, genes encoding antigenic polypeptides have been expressed in E. coli in order to identify and produce antigens involved with the development of protective immunity. Several clones expressing polypeptides recognized by sera which passively transfer resistance to reinfection have been identified and characterized. The importance of this shift towards molecular biology within the context of schistosome biology and immunology and the likely future impact of this approach will be discussed.

## *Molecular Paradigms for Evasion of Host Responses*

**C 015** IMMUNOREGULATORY CELLS AND MOLECULES IN HUMAN AND MURINE SCHISTOSOMIASIS, Daniel G. Colley, Veterans Administration Medical Center and Departments of Microbiology and Medicine, Vanderbilt University School of Medicine, Nashville TN 37203.

Human schistosomiasis is a chronic, intravascular infection which can result in severe, debilitating hepatosplenism, but more often leads to a stable, long-term, balanced relationship between the host and the parasite. At very low worm burdens most strains of mice mimic these overt clinical forms. It is proposed that the difference between clinical (hepatosplenic) and subclinical (intestinal) schistosomiasis rests largely on the host's ability to mount effective immunoregulatory responses in the face of continuous exposure to schistosomal antigens, in particular components of the soluble egg antigenic preparation (SEA). Multiple, potentially, immunoregulatory mechanisms, involving both cellular and molecular components, are expressed during the development of the chronic intestinal phase of schistosomiasis. In humans these have included: antigen-specific adherent cells; antigen-stimulated T suppressor cells; both T4+ and T8+ anti-idiotypic cells; histamine-receptor positive suppressor cells; antigen-specific suppressor activity in serum (serosuppression); soluble immune response suppressor (SIRS); and parasite-derived suppressor factors. In the murine model the observed immunoregulators encompass: T suppressor/inducer cells; T suppressor cells; histamine-receptor positive suppressor cells; I-J bearing, anti-idiotypic T suppressor factors; SIRS; anti-SEA monoclonal antibodies; and anti-idiotypic antibodies to major, cross-reactive, regulatory idiotypes. These regulatory activities have been most often measured in regard to their modulation of either lymphocyte proliferation or in vitro/in vivo granuloma formation, and may be reflected in the control of production of some lymphokines (Mitogenic Factor and Eosinophil Stimulation Promoter) during chronic infection. Recent studies have documented correlations between anti-SEA responsiveness, SIRS plasma levels, clinical forms, and chronicity, and have demonstrated anti-idiotypic T cells in the blood of chronic and former patients, and the cord blood of neonates born of mothers with active schistosomiasis.

Supported by the VA, AI-11289 and the World Health Organization.

**C 016** MOLECULAR MIMICRY REVISITED, Raymond T. Damian, Department of Zoology, University of Georgia, Athens, GA 30602

The theory of molecular or antigenic mimicry (Damian, R.T., 1964, Amer. Naturalist 98, 129; also papers by J.F.A. Sprent and J.K. Dineen) has influenced the development of immunoparasitology in that it added to the "special case" of antigenic variation (known only for African trypanosomes among parasites, at the time) to provide parasitologists and immunologists with a new generalization -- the avoidance of immune responses by parasites. A competing idea, antigenic disguise or masquerade (Smithers, S.R., R.J. Terry, and D.J. Hockley, 1969. Proc. Roy. Soc. Lond., Ser. B, 171, 483) is now thought to involve mimicry at a deeper level (Damian, R.T., 1979 in Nickol, B.B. (ed.) Host-Parasite Interfaces: At Population, Individual and Molecular Levels, Academic Press, N.Y.). This is through possible synthesis by parasites of specific receptors (e.g. parasite C3 and Fc-like receptors, Tarleton, R.L. and W.M. Kemp, 1981, J. Immunol., 126, 379) for acquiring a coat of host molecules. In contrast to the molecular basis for antigenic variation in trypanosomes, the basis for antigenic mimicry is less understood. As originally formulated (Damian, 1964), molecular mimicry was thought to reside primarily in similarity or identity of carbohydrate epitopes. Recent advances in the structural chemistry of oligosaccharides of glycoproteins and other glycoconjugates make possible the re-examination of numerous cross-reactions between parasites and host blood groups, as reported in the older literature. For example, our recent evidence suggests that Schistosoma mansoni synthesizes globoside, a glycosphingolipid which is the basis for human blood group P activity, as well as being the precursor of the Forssman antigen. Therefore, in appropriate host species and/or individuals, this may be an unrecognized (eclipsed) antigen or could stimulate autoimmunity. (Supported by NIH grant AI 18906 from the U.S.-Japan Cooperative Medical Sciences Program).

**C 017** PROTEIN PROTEINASE INHIBITORS FROM INTESTINAL PARASITIC HELMINTHS: STRUCTURE AND INDICATIONS OF SOME POSSIBLE FUNCTIONS, Robert J. Peanasky, Mark R. Martzen, Gene A. Homandberg and Donald R. Babin*, Department of Biochemistry, University of South Dakota School of Medicine, Vermillion, SD 57069 and *Department of Biological Chemistry, Creighton University School of Medicine, Omaha, NE 68178

Adult **Ascaris suum, Ascaris hominis,** and **Toxocara canis** live in the hostile degradative environment of the intestine. It has been postulated that these parasites possess a complement of antienzymes. They indeed do but these inhibitors may not simply inactivate host digestive enzymes in the intestine. Aqueous extracts of adult **A. suum** inhibit trypsin, chymotrypsin/elastase, carboxypeptidases A and B and pepsin. Isoforms of chymotrypsin/elastase, trypsin and carboxypeptidase inhibitors have been sequenced. Western Blot experiments have suggested some cross reactivity with the inhibitors of the other intestinal helminths.

The similar inhibitor profile of the intestinal parasites is distinct from that of free living **Caenorhabditis elegans.** This and the failure of trypsin inhibitor from **A. suum** to inactivate human trypsin, suggested the possible involvement of some inhibitors in species specificity. These experiments suggest other possible functions of inhibitors:

A. Live adult **Ascaris** were placed in a salt solution with fluorescein labeled chymotrypsin. Worms that were then fixed and sectioned showed fluoresence in the intestine, muscle and eggs/sperm of female/male **Ascaris** suggesting that the worms take up host proteins.

B. Antibodies against chymotrypsin/elastase inhibitors the trypsin inhibitor, and host environmental proteins, trypsin and chymotrypsin were used in a double antibody experiment as primary antibodies applied to cross-sections of adult **Ascaris.** Host enzymes and the inhibitors were visualized at the same sites as suggested by the uptake experiments. These host proteins seem to be taken up by live parasites as an inactive complex. Antibodies against both host and parasite antigens bind to the surface of the eggs.

C. Eggs naturally passed by **Ascaris** into saline picked up fluorescein labled chymotrypsin when this probe was incubated with the eggs (in the absence of the worm). These fluorescein covered eggs developed to L-2 stage larvae, and the fluoresence did not diminish. When naturally passed eggs developed to L-2 stage larvae under the same conditions, the double antibody technique showed the presence in larvae of host chymotrypsin and the chymotrypsin/elastase inhibitors. This and the finding that a chymotrypsin/elastase inhibitor can inactivate blood clotting Factor Xa may be associated with development of larvae. (Supported by NIH grant AI-10992.)

**C 018** GENETIC CONTROL OF VACCINE INDUCED IMMUNITY AGAINST SCHISTOSOMES, Alan Sher, Stephanie L. James, Rodrigo Correa-Oliveira, Paul J. Brindley, Dale McCall and Emil Skamene, Laboratory of Parasitic Diseases, NIAID, Bethesda, MD 20892, Departments of Medicine and Microbiology, George Washington University School of Medicine, Washington, DC 20037, Institute for Marine Biomedical Research, University of North Carolina at Wilmington, Wilmington, NC 28403 and Department of Immunology, Montreal General Hospital, Montreal, Quebec, Canada.

The genetic control of protective immunity to Schistosoma mansoni infection has been studied in an attempt to characterize the effector mechanism of attenuated vaccine induced resistance and to define genetic loci regulating this process. Of all inbred mouse strains surveyed, the P and A (A/J) strains developed the lowest levels of resistance to challenge infection after vaccination with irradiated cercariae. Genetic cross experiments as well as recombinant inbred strain surveys indicate that the protective immunity defects of these two strains are controlled by single yet distinct loci unlinked to either the murine H-2 or immunoglobulin heavy chain loci. Vaccinated P and A strain mice were also found to be defective in their capacity to produce activated macrophages which kill schistosome larvae in vitro. In the case of both strains, an association of this macrophage larvicidal defect with defective vaccine induced resistance was observed in backcross or $F_2$ progeny produced by matings of A or P mice with animals of the highly resistant C57BL/6 strain. No other congenital defects in humoral or cellular immune response have been detected which genetically correlate with impaired protective immunity. These results support the hypothesis that T-lymphocyte dependent macrophage activation plays a crucial role in the effector mechanism of anti-schistosome immunity. Since P and A strain mice have previously been shown to be defective in their resistance to other infections, it is possible that the loci determining the impaired protective immunity of these strains to schistosomes may play a general role in the regulation of host susceptibility.

## *Biochemical Paradigms for Rational Chemotherapy*

**C 019** BIOCHEMICAL TARGETS FOR CHEMOTHERAPY OF TREMATODES, Chris Bryant, Department of Zoology, Australian National University GPO Box 4, Canberra, ACT 2601, Australia.

It is clear that protective vaccines against helminths are far in the future. The complexity of helminths and our incomplete knowledge of all aspects of their life histories are important factors that militate against a quick immunological solution to the problem of human and animal helminthiases (1). The corollary of this observation is that the chemotherapeutic arsenal for the treatment of parasitic diseases is of paramount importance in the short and middle terms at least and, in the long term, will certainly have a role to play as support for the immunological approach. In several host-parasite interactions it has already been shown that anthelmintics and the immune system can interact synergistically to increase anthelmintic efficacy. Unfortunately for chemotherapy, the trematodes do not appear to be a biochemically homogeneous group. On the one hand there are the schistosomes, that have been shown recently to have a far more complex metabolism than had hitherto been suspected - for example, the concept of homolactate fermentation as the sole energy producing pathway is now suspect, and recent studies have shown a dynamic biochemical relationship between males and females. On the other hand, there are the flukes occupying other environments, of which **Fasciola** is by far the best known example. Comparisons of the two sorts of trematodes at the biochemical level do not suggest homogeneity. In a recent review (2) of the modes of action of some modern anthelmintics, Behm and Bryant (1985) followed earlier workers in classifying them as (i) those that exploit the differences in neurophysiology between host and parasite, (ii) those that interfere with essential energy metabolism, (iii) those that affect essential biosyntheses and (iv) those that interfere with essential cellular structures. All existing chemotherapeutic agents against trematodes act by a combination of effects from more than one of these categories, and all are thus potentially injurious to the host. The efficacy of future anthelmintics will depend as much on new techniques of delivering the drug to the parasite and otherwise manipulating its pharmacokinetics as on the identification of exquisitely specific biochemical targets within the parasite.

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2. Behm C.A. and Bryant C. (1985). The modes of action of some modern anthelmintics. In Resistance of Nematodes to Anthelmintic Drugs (edited by N. Anderson and P.J. Waller) pp 57-68, Commonwealth Scientific and Industrial Research Organisation, Australia.

**C 020** MITOCHONDRIAL TRANSHYDROGENATION AND ANAEROBIC ENERGY GENERATION BY PARASITIC HELMINTHS, Carmen F. Fioravanti, Bowling Green State University, Bowling Green, OH 43403.

The adult parasitic helminths are characterized by their incomplete dissimilation of glucose with accompanying organic acid end-product accumulation. Numerous helminths accumulate succinate, or products derived from succinate, and carbohydrate-dependent succinate formation is the result of mitochondrial events that occur anaerobically. In these systems, malate is the substrate for an intramitochondrial dismutation reaction. The oxidative portion of the dismutation is catalyzed by a "malic enzyme" that yields reduced pyridine nucleotide. Via mitochondrial fumarase, malate also is converted to fumarate. The dismutation is completed with the reduced pyridine nucleotide-dependent, electron transport-associated reduction of fumarate to succinate, thereby permitting a physiologically required, site I-dependent anaerobic generation of ATP. Amoungst the helminths, two types of mitochondrial "malic enzyme" are apparent, viz., an NADP-specific type or a type that preferentially uses NAD. The predominantly anaerobic, succinate-forming adult Hymenolepis diminuta model represents those helminths that employ an NADP-specific "malic enzyme". Thus, in H. diminuta, reducing equivalents for electron transport-coupled succinate accumulation and anaerobic phosphorylation are generated in the form of NADPH. However, the cestode's fumarate reductase requires NADH. Accordingly, in H. diminuta and other helminths, a mechanism that catalyzes hydride transfer from NADPH to NAD is crucial to the malate dismutation. This need is fulfilled by an NADPH→NAD transhydrogenase and H. diminuta mitochondria catalyze this transhydrogenation. The H. diminuta NADPH→NAD system is membrane-associated and couples NADPH utilization with electron transport. This transhydrogenase is phospholipid-dependent, differs from the electron transport mechanism based on lipid requirement, is bound to the inner membrane and apparently catalyzes a scalar hydride transfer on the matrix surface of this membrane. Kinetic evaluations of the transhydrogenase indicated two substrate-binding domains (i.e., an NADP(H) and NAD(H) domain) and the occurrence of site-specific and end-product inhibition. H. diminuta submitochondrial particles catalyzed the reversible NADPH→NAD transhydrogenation and an "energy-linked" NADH→NADP reaction that required $Mg^{++}$ and ATP. This suggested a relationship of the transhydrogenase with membrane energization in the helminths. Given the energetic implications of the helminth NADPH→NAD transhydrogenase, further studies are warranted particularly within the context of vulnerability to specific chemotherapeutic disruption

Supported in part by NIH Grants AI-15597, AI-00389 and a Research Challenge Grant, Ohio Board of Regents.

**C 021** APPLICATIONS OF NMR TO INVESTIGATION OF METABOLISM AND PHARMACOLOGY OF PARASITES, Paul M. Matthews and Tag E. Mansour, Stanford Medical School.

We are studying unique features of glycolysis in parasitic trematodes and developing improved methods for monitoring effects of pharmacologic agents on this pathway. Nuclear magnetic resonance (NMR) spectroscopy is a powerful technique to study the biochemical physiology and pharmacology of a variety of cells and tissues (1). F. hepatica can be maintained in the spectrometer for extended periods, allowing free, cytosolic metabolite concentrations and intracellular pH to be monitored. In addition, NMR can provide information on compartmentation of metabolites between different cell types, enzyme systems, and between free and macromolecule-associated species. We used Varian XL-40 or XL-300 spectrometers and a perfusion chamber that provided for circulation of warmed (37°) medium. The $^{31}P$ NMR spectrum of the worm showed unusual metabolic features, among which are large amounts of glycerolphosphorylcholine, phospholipids mobile on the NMR time-scale, and concentrations of free cytosolic ADP an order of magnitude higher than in mammalian tissues (2). The intracellular pH of 6.97 $\pm$ 0.05 is lower than in mammalian tissues and reversibly decreases to as low as 6.12 after glycolytic stimulation with serotonin. $^{13}C$ NMR was used to study the metabolism of $^{13}C$-labeled substrate (3). [1-$^{13}C$] glucose is incorporated into glycogen at 11% of the glycolytic rate of 12 µmol glucose/g wet wt/hr and is metabolized to the end-products propionate and acetate in a fixed ratio of 2.2 $\pm$ 0.1. Observation of $^{13}C$-induced multiplet splitting of adjacent protons by $^{1}H$ NMR allowed calculation of the quantitative enrichment in $^{13}C$ at each site in the end-products, demonstrating that acetate is generated from pyruvate formed by the malic enzyme reaction rather than more directly from cytosolic phosphoenol pyruvate. Regulation of glycolytic flux occurs via enzymes such as phosphofructokinase, as well as by changes in effector metabolite (e.g., ADP, ATP, $P_i$) levels. Alterations in glycolytic flux achieved after modest effector concentration changes following stimulation with serotonin may be due to a fructose-6-P/fructose-1,6-$P_2$ "substrate cycle" (4) that enhances regulatory sensitivity at the cost of increased energy demand. Evidence for such cycling came from incorporation of glucose equivalents labeled at both C-1 and C-6 positions into glycogen during perfusion with [1-$^{13}C$] glucose. From the observed glycogen C-6/C-1 labeling ratio of 0.42, it was calculated that total flux through this cycle can be almost three times net glycolytic flux. Substrate cycling consumes ~25% of the total ATP production at low rates of glycolysis. We will emphasize that the techniques employed in our work are applicable to a broad range of problems and biological systems, and employ instrumentation now widely available. (Supported by USPHS Grant AI 16501.)

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## *Chemotherapy: Molecular, Cellular, Organismic, Ecological*

**C 022** PRAZIQUANTEL: SELECTIVE KILL OF TREMATODES AND CESTODES BY DISRUPTION OF TEGUMENTAL INTEGRITY, Peter Andrews, Institute of Chemotherapy, Bayer AG, D 5600 Wuppertal 1, P.O.Box 101709, FRG.

Praziquantel (PZQ) is effective against trematodes (e.g.: Schistosoma, Paragonimus, Clonorchis, Opisthorchis, Metagonimus, Heterophyes) of man and animals. Parasites living in the blood are almost equally susceptible to the drug as those in the intestine, lung or liver. All adult cestodes (e.g.: Taenia, Hymenolepis, Diphyllobothrium, Mesocestoides) and many larval cestodes can be successfully treated with PZQ.

The pharmacokinetics of PZQ are reviewed as a basis for understanding mode of action studies. Two striking phenomena can be observed in schistosomes exposed to PZQ within 30s: an almost instantaneous spastic paralysis and damage to the tegument. The threshold concentration of PZQ is 0.32 to 0.6 µM, consistent with plasma concentrations measured in man.

Most inhibitors of known neurotransmitters of Schistosoma mansoni and other pharmacologically active agents do not antagonise the action of PZQ. PZQ does not act as a $Ca^{++}$-ionophore. However, fluoxetine (FX) was found to attenuate the PZQ-induced $Ca^{++}$-influx as well as contracture. FX and other amphiphilic cationic drugs fluidize acidic phospholipid membranes, replace $Ca^{++}$ from membranes and inhibit membrane fusion. This lead to the hypothesis that PZQ interacts with tegumental phospholipids. To support this idea, the effects of amphiphilic cationic drugs and PZQ on the carbohydrate metabolism of Schistosoma mansoni, which requires intact membranes for optimal functioning, were compared.

The amphiphilic cationic drugs stimulate glucose uptake and lactate excretion at 10 and 100 µM but inhibit at 1 mM. PZQ on the other hand stimulates at 0.1 µM and inhibits above 1 µM. 10 µM PZQ and FX inhibit $Na^{+}$-sensitive serotonin uptake and excretion. Glycogen breakdown is maximally stimulated by 1µM PZQ. FX and PZQ both affect the stimulation of carbohydrate metabolism by serotonin. Amphiphilic drugs and PZQ may influence the carbohydrate metabolism by interfering with the same binding sites. With respect to alterations of membrane properties there are some important differences between the electrically neutral PZQ and positively charged amphiphilic drugs. The latter replace $Ca^{++}$ from membranes, reduce membrane bound $Ca^{++}$, and cause flaccid paralysis. PZQ enhances $Ca^{++}$ permeability and causes spastic paralysis.

The interaction of phospholipis with PZQ and FX was also studied directly. FX fluidizes a synthetic phosphatidylglycerol membrane, PZQ has only little effect. FX does not induce fusion of synthetic phospholipid vesicles, while PZQ induces aggregation and fusion. The resulting $Ca^{++}$-influx will disrupt various regulation processes triggered by $Ca^{++}$ and this then leads to the death of the parasite.

**C 023** AVERMECTINS VS. NEMATODES, William C. Campbell, Merck Institute for Therapeutic Research, P. O. Box 2000, Rahway, NJ 07065. Avermectins are active against nematodes of the following families: Ascarididae, Oxyuridae, Rhabditidae, Strongyloididae, Strongylidae, Stephanuridae, Ancylostomatidae, Trichostrongylidae, Dictyocaulidae, Metastrongylidae, Protostrongylidae, Filaroididae, Spiruridae, Thelaziidae, Filariidae, Setariidae, Onchocercidae, Trichinellidae, Trichuridae, Capillariidae. Two avermectins, ivermectin and avermectin $B_1$, are in commercial use. To the extent that the two drugs have been tested against the same species, there is no known difference in spectrum of efficacy. The avermectin dosage required for efficacy varies with the species and developmental stage of nematode involved. The dosage of ivermectin currently recommended for broad-spectrum use is 0.2 mg/kg for sheep, cattle and horses, and 0.3 mg/kg for swine. Avermectin $B_1$ is used in cattle at 0.2 mg/kg.

# Molecular Immunoparasitology

**C 024** ANTI-IDIOTYPES AND VACCINES AGAINST SCHISTOSOMIASIS, A. CAPRON, J.M. GRZYCH, J.M. BALLOUL, C. DISSOUS, M. CAPRON and R.J. PIERCE, Centre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, Lille, France.

Significant progress has recently been made in the characterization, molecular cloning and chemical synthesis of two major immunogens of S. mansoni schistosomulum surface clearly implied in protective immunity.

A surface 38.000 MW glycoconjugate was initially characterized by a protective rat IgG2a monoclonal antibody (IPLSm1). Various experiments confirmed the carbohydrate nature of the protective epitope and led to a strategy of antiidiotype immunization. Anti-idiotype antibodies (AB2) were produced against IPLSm1 (AB1). Immunization of rats with AB2 led to the production of specific (anti-38 kD) and highly cytotoxic and protective antibodies and more importantly induced a high degree of protection to challenge infection in recipient animals.

More recently, we have demonstrated that the protective carbohydrate epitope was expressed on various fresh-water and marine snails (Nature, 1986, 323, pp. 443-448). A total identity was in particular demonstrated between the carbohydrate of the 38 kD schistosome molecule and the oligosaccharide of key hole limpet hemocyanin. Immunization with KLH led to the production of anti-schistosome cytotoxic and protective antibodies and induced a high degree of protection. Purification of the oligosaccharide has been achieved and chemical analysis performed by mass spectrometry and NMR. The vaccine potential of this molecule will be considered.

Another major immunogen P28 has recently been cloned and expressed in various vectors. Immunization with the biosynthetic protein has resulted in significant protection in rats, mice and hamsters.

The access which we now have to protein and carbohydrate protective epitopes of schistosomes opens interesting prospects of constructing neoglycoproteins that can be used as entirely synthetic vaccines.

**C 025** CANDIDATE EPITOPES FOR VACCINATION AGAINST SCHISTOSOMIASIS MANSONI, Donald A. Harn, Lyn Oligino, Albert Ko, and Lincoln D. Stein, Dept. of Tropical Public Health, Harvard School of Public Health, and the Dept. of Medicine, Harvard Medical School, Boston, MA 02115.

We have identified a number of murine, monoclonal antibodies which are able to passively transfer resistance to cercarial challenge in mice. As probes, these antibodies have been used to characterize three, distinct, surface membrane antigens as candidates for the development of a defined vaccine in schistosomiasis, (1-3). The antigens have approximate molecular weights of: 1), 22 kD; 2), 28 kD; and 3), a >200, 160, 38 kD complex. All of the antigens have been purified using either immunoaffinity chromatography or electro-elution. Antisera from rabbits immunized with the purified antigens immunoprecipitate the native molecules as well as the correct molecular weight in vitro translation products.

We have determined that the epitope on the >200, 160, 38 kD complex is a surface membrane component of sporocysts, cercariae/schistosomula, and the ciliary plates of miracidia (4). Although the antigen persists on parasites in vitro, it rapidly disappears in vivo. Passive transfer of immunity experiments suggest that this antigen is only relevant to early phase killing (4). We have determined that there are 3-4 carbohydrate epitopes on this molecule. Some of the epitopes are shared with the glycan epitopes of KLH.

We have immunized mice with very small amounts of the purified antigens, and have the following initial results: immunization with the 22 kD (200ng - 1ug), gave 15 - 27% resistance; 500 ng of the >200, 160 38 kD complex gave 35% resistance; and immunization with 2 - 5 ug of the 28 kD antigen gave 38% resistance. All of the mice were challenged with cercariae 4 - 5 weeks after the boost; indicating that these three antigens were able to induce a protective memory response.

We are currently analyzing recombinant clones for all three antigens. The recombinants were selected using rabbit antisera to the purified antigens.

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C 026 IDENTIFICATION AND CLONING OF S. MANSONI SCHISTOSOMULAR SURFACE ANTIGENS DETECTED BY PROTECTIVE ANTIBODIES, Mette Strand, John P. Dalton, Timothy D. Tom, Michael S. Roberts, Johns Hopkins University, Department of Pharmacology and Molecular Science, 725 N. Wolfe Street, Baltimore, MD 21205.

Using radiolabeling, immunoprecipitation and immunodepletion techniques, we have identified a number of adult and schistosomular antigens which elicit an enhanced or unique response in mice vaccinated with radiation-attenuated cercariae, as compared to patently infected mice. These "vaccine-specific" antigens were used to raise antisera (anti-IrV) in rabbits; a monospecific rabbit antiserum (anti-654) was also prepared against an 18-kDa polypeptide identified on the surface of schistosomula by monoclonal antibody 654. The anti-IrV and anti-654 antibodies are of particular interest because they recognize epitopes expressed on the surface of lung-stage schistosomula, as has previously been demonstrated for all sera capable of transferring resistance.

In order to obtain the large quantities of schistosome antigens necessary for active vaccination experiments, we have constructed an adult worm cDNA expression library in λgt11, and have screened this library with anti-IrV and anti-654, as well as with sera from vaccinated mice. Numerous immunoreactive cDNA clones have been identified, of which five have been subcloned into a dual promoter transcription vector and their coding strands identified. We have also begun to identify the native protein homologues of the recombinant gene products, using antisera raised against lysates of bacteria infected with the recombinant phages. Four glycoproteins, of 18, 38, 100 and 200 kDa, have thus far been detected by such anti-fusion protein antisera. All of these are expressed on the surface of schistosomula, and are reactive with sera of vaccinated mice. The ability of the fusion proteins to elicit protective immunity against cercarial challenge is being examined in the mouse model.

## *Receptors, Transducers and Neuroparasitology*

**C 100** CARBON-13 NMR STUDIES OF ENERGY METABOLISM IN *HAEMONCHUS CONTORTUS*. Carolyn A. Behm, C. Bryant & S. Kohlhagen, Zoology Department, Australian National University, Canberra 2601, Australia.

Adult *H. contortus* were incubated *in vitro* with $NaH^{13}CO_3$, U-$^{13}$C-glucose, or 1-$^{13}$C-propionate under aerobic conditions. The incubation media and perchloric acid extracts of the worms were analysed at 50.309 MHz by FT-NMR. Glucose is converted to the major end products 1-propanol, propionate and acetate; the internal malate and succinate pools in the extracts are clearly labelled. $H^{13}CO_3$ is incorporated extensively into propanol C-1, propionate C-1 and succinate C-1. The labelling patterns observed are consistent with glucose catabolism via glycolysis, with $CO_2$ fixation contributing to the malate and succinate pools. The retention of label from bicarbonate in the C-1 of propanol and propionate indicates that the "forward" TCA cycle reactions do not contribute substantially to the succinylCoA/propionylCoA pools from which these end products are derived. 1-$^{13}$C-propionate is converted by the worms to 1-$^{13}$C-propanol, though it is not clear at present whether this is due to a net conversion or to equilibration within the pathways. We do not understand what role the production of propanol plays in the metabolic economy of *H. contortus*; this end product is produced only by certain trichostrongylid nematodes of ruminants. Elucidation of the synthetic pathway for propanol and characterisation of the enzymes responsible may provide new potential sites for chemotherapeutic attack.

**C 101** THE ABSORPTION AND SYNTHESIS OF 5-HYDROXYTRYPTAMINE IN THE MUSCLE AND INTESTINE OF ADULT ASCARIS SUUM, Manus J. Donahue, Rex E. Martin, and Jayanta Chaudhuri, North Texas State University, Denton, TX 76203.

Serotonin (5-hydroxytryptamine, 5-HT) has been proposed as a putative hormone in adult Ascaris suum because it causes activation of glycogenolysis and a dose dependent increase in cyclic AMP, an activation of phosphorylase and an inactivation of glycogen synthase. The origin of 5-HT in A. suum is not known. Evidence is presented here in which 5-HT has been histochemically localized in the muscle and intestinal tract of female A. suum using fluorescent microscopy. Serotonin receptors (5-$HT_2$) were identified in the muscle and intestinal tract of these worms. The $IC_{50}$ for mianserin (an effective competitor for 5-HT analogues) was 1 X $10^{-3}$ M for intestine and 2 X $10^{-9}$ M for muscle. When tissue was incubated with 2.3 nM ($^3$H)-LSD + 100 µM mianserin, the specific binding for muscle and intestine was respectively, 435 (±39) and 255 (± 14) pmoles ($^3$H)-LSD bound per g membrane protein. The $K_D$ for muscle and intestine respectively was 3.7 nM and 4.4 nM. When isolated muscle and intestinal tract were perfused with varying concentrations of tryptophan (TRP) and the metabolites of 5-HT were extracted and isolated (TRP, 5-hydroxy-tryptophan, 5-HT, 5-hydroxyindole acetic acid, and 5-hydroxytryptophol) and analyzed using high performance liquid chromatography a dose dependent increase in the metabolites of 5-HT were observed up to a concentration of $10^{-5}$ M. Collectively the data indicated that 5-HT may be absorbed from the intestine of the host by the parasite or that 5-HT may be synthesized de novo in the parasite's tissues. (Supported by NIH grant AI-22479).

**C 102** ***THE BIOPHYSICAL PROPERTIES OF THE SURFACE LIPID OF MICROFILARIAE ARE DIFFERENT FROM OTHER NEMATODES.*** Malcolm W. Kennedy, Michael Foley, John R. Kusel and Peter B. Garland. Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Glasgow G61 1QH, Scotland, U.K., and the Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, U.K.

The outer surface of parasitic nematodes is composed of a lipidic structure termed the epicuticle, which comprises the host-parasite interface. We have studied the biophysical properties of this layer using fluorescent lipid analogues and Fluorescence Recovery After Photobleaching (FRAP), and have found unique features which distinguish it from the classical cellular plasma membrane. These were typified by the infective larvae of Toxocara canis, and all the life-cycle stages of Trichinella spiralis, in which the surface lipid is unusually selective for the insertion of lipid probes, and in which the lateral diffusion of inserted probe within the plane of the epicuticle is highly restricted. Analysis of the rodent filarials Dipetalonema viteae and Litomosoides carinii revealed similar properties of the epicuticle of adult worms of both species, and the microfilariae of the former. In contrast, the surface of L. carinii microfilariae, within their sheath, was remarkably different. This parasite showed no surface-specific selectivity for the probes used, many of which could only penetrate below the epicuticle of these microfilariae. Moreover, treatment with the hydrophobic vital DNA stain Hoechst 33342 showed penetration in both D. viteae and L. carinii microfilariae, but in no other nematodes examined. Thus, the biophysical properties of the surface lipid of microfilariae appear to differ significantly from other nematodes, and sheathed microfilariae in particular have a surface which is a highly specialised form of this unique structure.

**C 103** EVIDENCE THAT AEROBIC GENERATION OF ENERGY IN S. Mansoni IS NOT PHYSIOLOGICALLY RELEVANT. C. Lane, L. Foster, R. Pax and J. Bennett. Department of Pharmacology, Michigan State University, East Lansing, MI 48823. The concept that adult S. mansoni is a homolactic fermenter (Bueding, 1950; J. Gen. Physiol.) continues to be questioned. Recently it was demonstrated that up to 30% of the parasite's energy is derived, when incubated in a simple salt medium containing glucose, from aerobic metabolic processes (Van Oordt et al., 1985; Molec. Biochem. Parasit.). The authors of this work readily admit that a simple salt medium is physiologically stressful to the parasite but to reveal the aerobic pathway they were forced into using this media. The most important question which remains to be answered is, what is the physiological significance of this aerobic generation of energy? To answer this question we incubated adult schistosomes for 24 h in a similar simple salt medium containing (1) no organic constituents (2) 10mM glucose (3) 10mM glutamine or (4) 10mM glucose and glutamine and then measured parasite muscle function and transmembrane potential - two physiological processes dependent upon parasite energy levels. Results demonstrated that ATP concentration, muscle function and transmembrane potential were markedly reduced in parasites kept in media with no organic constituents while these 3 parameters were not affected in parasites maintained in the other 3 media. Parasites in the other 3 media were then exposed to 3 μ M rotenone, 3 μ M antimycin-A, 1mM cyanide or 1 μ M oligomycin. ATP levels, muscle function and the transmembrane potential of schistosomes maintained in glutamine were all significantly depressed within 5-10 min while parasites maintained in glucose or in glutamine plus glucose were unaffected. These results suggest that adult schistosomes can survive in a media containing glutamine and that this substrate generates energy aerobically but if glucose is present the parasite can function without aerobic generation of energy.

**C 104** IDENTIFICATION OF TUBULIN ISOFORMS FROM BRUGIA MALAYI, B. PAHANGI AND NIPPOSTRONGYLUS BRASILIENSIS, AND COMPARISON WITH MAMMALIAN BRAIN TUBULIN, Liang Tang and Roger K. Prichard, McGill University, Montréal, Qué., Canada H9X 1C0, and Ernest Lacey, CSIRO, McMaster Laboratory, Sydney, Australia.
Comparison of ($^3$H)colchicine and($^3$H)benzimidazole binding to rat brain tubulin and parasite (B.malayi, B. pahangi and N. brasiliensis)tubulin show that ($^3$H)colchicine has high binding activity to mammalian tubulin and less binding activity to parasite tubulin. The ($^3$H)benzimidazoles, however, have high binding activity to parasite tubulin. All of these tubulins have very similar electrophoretic migration on the SDS polyacrylamine gel. Mammalian brain tubulin and parasite tubulin were purified by poly-L-lysine gel and concentrated. Tubulin rich fractions were identified by ($^3$H)colchicine and ($^3$H)BZs binding assays, and SDS polyacrylamine gel electrophoresis. Different isoforms of both mammalian brain tubulin and parasite tubulin were investigated by using isoelectrofocussing and two-dimension electrophoresis methods. The isoforms were identified by peptide mapping and immunoblotting.

**C 105** Acetylcholinesterase of Schistosoma mansoni: Implications. Rebeca Tarrab-Hazdai, Bert..a Espinoza-Ortega and Ruth Arnon. Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot.
The presence of acetylcholinesterase (AChE) in S. mansoni is known since 1952. We have previously shown that this enzyme exists in all the life stages of the parasite, is particularly enriched in membrane preparations. This enzyme is cross-reactive with rabbit antibodies raised against AChE from electric eel, which have a cytotoxic effect on the parasite. AChE is generally required at the cholinergic synapsis and thus could be envisaged as a target for anti-functional activity. The enzyme was purified on an affinity column to which specific inhibitors was bound. Elution from this column yielded a material which migrated as a single protein band of 450 KD in non-reducing non-denaturing polyacrylamide gel electrophoresis (PAGE). Analysis in SDS-PAGE in the presence of β-mercaptoethanol revealed two major polypeptide components of approximately 70 KD and 30 KD and a minor one around 45 KD. By labelling with $^3$H-DFP, the 30 KD polypeptide was identified as the catalytic subunit of the enzyme. Antibodies against the enzyme were raised in rabbits. In immunoprecipitation with the total $^{125}$I-labelled extracts these anti-AChE antibodies revealed three polypeptide bands of 35 KD, 32 KD, 12 KD and a minor one of 45 KD. The 32 KD band is the only one that immunoprecipitates from $^3$H-DFP labeled shistosome extract. In immunofluorescence microscopy anti-AChE antibodies showed specific staining of intact schistosomula. Moreover, they led to a substantial complement dependent cytotoxicity towards the parasite larvae (87-95% killing). These results emphasize the potential of AChE for immunization purposes.

**C 106** CHARACTERIZATION OF PORPHYRINS -$Zn^{++}$ ISOLATED FROM INTRACYST LIQUID IN CYSTICERCUS CELLULOSAE. Martínez Zedillo, G., Research Department IMSS-CMN, 73032 México 73, D.F.

Derrien in 1927 describe fluorescent compounds in the intracyst liquid from metacestode of Taenia solium. During long time the fluorescence has was used diagnostic test in porcine and bovine cisticercosis. However the estructure and properties of these substances remains unknow, the present paper using methods: spectrofluorometric, electrophoresis and thin layer chromatography demostrate the presence of 3-4 porphyrins $Zn^{++}$ (15-48 mcg) with 5 carboxilic acid side chains. The demostration of this singular porphyria require systematic study for clarify the biosyntetic pathways and biological function in the parasite, and the role in pathogenic host-parasite relationship, the analysis of this tetrapyrroles or yours metabolites represent a potential diagnostic test and the photoexcitable capacity could be use for selective parasite destruction.

## *Clones, Hybridomas and Chimeras*

**C 200** GENOMIC CLONING OF RECOMBINANT ANTIGENS DETECTED BY ANTISERUM AGAINST INFECTIVE LARVAE OF *B. malayi*. Prema Arasu, Mario Philipp and Francine B. Perler, New England Biolabs, Inc., Beverly, MA 01915.

A *B. malayi* genomic expression library was screened with rabbit antiserum generated against live infective larvae (L3) of the parasite. From positively identified recombinant clones, a profile of antigenic reactivities was compiled by cross-analyses with antisera raised against various filarial parasites (*Brugia pahangi, Onchocerca volvulus* and *Dirofilaria immitis*), human serum pools from Indians infected with *B. malayi* and various individual serum samples from Indonesians with and without detectable levels of circulating microfilariae of *B. malayi*. Three broad categories of recombinant clones were identified encoding (1) antigens that were cross-reactive with all antisera tested, (2) antigens that were specific to the *Brugia* genus, and (3) antigens that appeared to be specific to *B. malayi*. Clones of the latter category were further analyzed for stage and species specificity. One such example is λBm 22 which encodes a 124 kdal fusion protein; the single 2.3 kb *Eco*RI insert is unique among the group of 32 other recombinant phage clones. In Western blot analysis, λBm 22 is recognized by antisera against *B. malayi* and also *Wuchereria bancrofti*, but not by antisera against *B. pahangi, O. volvulus* or *D. immitis*. Mouse antiserum generated against the fusion protein of λBm 22 recognized a single 220 kdal protein in *B. malayi* antigen extracts from both the adult and L3 stages. Further analyses will be conducted to evaluate the immunodiagnostic potential of λBm 22. In addition, experimental model systems of filariasis will be used to study the protective capacity of selected recombinant antigens including clones expressing proteins that are recognized by antibodies in human sera from amicrofilaremic but not by microfilaremic individuals.

**C 201** ISOLATION AND CHARACTERIZATION OF A cDNA CLONE PREDOMINANTLY EXPRESSED IN ADULT MALE SCHISTOSOMA MANSONI, David Bieber, Mario Zurita, Gordon Ringold and Tag E. Mansour. Department of Pharmacology, Stanford University, Stanford CA 94305.

Male and female schistosomes were separated and a cDNA library from each sex constructed in lambda gt10 vectors. Differential screening of these libraries produced cDNA clones of mRNAs preferentially expressed in males or females. One of these male clones is ~ 500 bp in length and was characterized in more detail. Northern analysis demonstrates that the mRNA of ~ 2 kb is expressed in adult males at a level approximately 10-fold greater than in females. This message is not detected in miracidia or cercariae. The onset of expression at various stages of somule development or in early adults is currently under investigation. This male-abundant RNA is not present in other trematodes like Fasciola hepatica or Echinostoma paranensi. Experiments to identify the protein product of this gene and to elucidate its genomic structure are underway. (Supported by a grant from the MacArthur Foundation.)

**C 202** ISOLATION AND PARTIAL SEQUENCE ANALYSIS OF AN "ADULT-FEMALE-SPECIFIC" GENE FROM THREE SPECIES OF HUMAN SCHISTOSOMES, Libuse A. Bobek, David M. Rekosh and Philip T. LoVerde, SUNY at Buffalo, Buffalo, New York 14214.

Previously we isolated and characterized a cDNA clone derived from a developmentally regulated mRNA found only in adult females of Schistosoma mansoni (Proc. Natl. Acad. Sci.(USA) 83:5544, 1986). The deduced sequence of the polypeptide encoded by this clone had significant homologies to the silk moth chorion (egg shell) protein gene family.

We have now used this cDNA clone as a hybridization probe to screen genomic libraries of S. mansoni, S. haematobium and S. japonicum. (These libraries were constructed by cloning partial Sau3A digests of genomic DNAs into the BamHl site of the bacteriophage lambda EMBL3.) Several recombinant phages (genomic clones) were isolated from each library. These are being further characterized by restriction enzyme mapping and DNA sequencing. A comparison of the different isolates from each species will be presented and their relationship described.

**C 203** TRICHINELLA SPIRALIS: CLONING STAGE SPECIFIC ANTIGEN GENES,
Anne E. Chambers, Matty Knight, Charles Kelly, Andrew J.G. Simpson and R. Michael E. Parkhouse.

Several gt11 and Pex2 cDNA clones encoding antigenic polypeptides specific to the infective larval stage of Trichinella spiralis have been identified using a spectrum of defined antisera.

One of these clones is identified by a polyclonal antiserum raised against a purified protective surface component.

Progress in the characterisation of these cDNA clones at both the immunological and nucleic acid level is presented.

**C 204** ISOLATION OF *SCHISTOSOMA MANSONI* λgt11 cDNA CLONES THAT ARE COMPLEMENTARY TO MOUSE HYPOXANTHINE-GUANINE PHOSPHORIBOSYL-TRANSFERASE cDNA, Sydney P. Craig, James H. McKerrow, and Ching C. Wang, University of California, San Francisco, CA 94143

*Schistosoma mansoni* possesses the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) for the salvage of purines (Dovey *et al.* 1986, J. Biol. Chem. **261**, 944) but lacks enzymes for *de novo* purine nucleotide synthesis (Senft *et al.* 1972, Int. J. Parasit. **2**, 249 and Dovey *et al.* 1984, Mol. Biochem. Parasit. **11**, 157). Thus, the HGPRTase may be an excellent target for antischistosomal chemotherapy. In an effort to determine details of the structure of the parasite enzyme for specific inhibitor design, we have opted to isolate and sequence the *S. mansoni* cDNA encoding HGPRTase. For this purpose we used the pHPT5 plasmid containing the complete cDNA of the mouse HGPRTase (generously provided by T. Caskey of Baylor). Since previous efforts to hybridize this probe with *S. mansoni* DNA were unsuccessful (Dovey *et al.* 1986, J. Biol. Chem. **261**, 944), the cDNA was nick translated and used at very relaxed stringency (37° in 5X SSC, 30% formamide, etc.) to identify complementary sequences within restriction fragments of genomic DNA of *S. mansoni* resolved in Southern blots. The results showed that the complementary sequences are in a single Pst I fragment approximately 1400 base pairs in length with a single Hind III site and no Eco R1 sites. The same probe was used to screen a lambda gt11 *S. mansoni* cDNA library (kindly provided by D. Lanar of the N.I.H.). Roughly 50,000 phage clones were screened, yielding two positive plaques which were subcloned twice to apparent homogeneity. The two independently isolated phage clones were lysogenized into *E. coli* strain Y1089. Current efforts are focused on subcloning the *S. mansoni* cDNA's into Bluescript (from Stratagene) and pKK 233 for nucleotide sequencing and gene expression, respectively.

**C 205** A MAJOR IMMUNOGEN IN Schistosoma mansoni INFECTIONS IS HOMOLOGOUS TO HEAT SHOCK PROTEIN HSP 70, Janice A. Culpepper, Richard C. Hedstrom, Robert A. Harrison, Nina M. Agabian, and George R. Newport, Jr., University of California, Berkeley, Naval Biosciences Laboratory, 140 Warren Hall, Berkeley, CA 94720 USA.

We have recently identified a major antigen in S. mansoni infections as being homologous to the heat shock protein hsp 70. A cDNA encoding this 70 kd protein was isolated from a λgt11 expression library by screening with various infection sera. The cDNA insert was analyzed by DNA sequencing and the amino acid sequence deduced was found to be highly homologous to that of human, Drosophila, and E. coli hsp 70. However, the sequence is sufficiently divergent to elicit a strong cross-reactive immune response in host animals. The antigen appears to be highly expressed in S. mansoni as judged by SDS-PAGE and immunoblot analysis with adult worm and cercariae extracted proteins. Heat shock or stress induce the production of hsp 70 in many species; however, in S. mansoni the expression of both the 2.2 kb mRNA encoding hsp 70 and the protein itself appears to be constitutive. Genomic southern analysis suggests that the gene is organized in tandemly repeated copies in the genome. Characterization of genomic clones of S. mansoni hsp 70 are currently underway.

**C 206** MOLECULAR CLONING OF SCHISTOSOMA MANSONI HEMOGLOBINASE, Alan H. Davis, Jayasri Nanduri, Doug Watson, and Ron Blanton, Case Western Reserve University, Cleveland, OH 44106.

Schistosoma mansoni consumes host red blood cells and utilizes an acidic, thiol hemoglobinase for degradation of host hemoglobin. The native enzyme was purified from adult worms by gel filtration and affinity chromatography on agarose-phe. A specific rabbit antiserum against the purified enzyme was utilized to isolate cDNA clones from an expression library in λgt11 amp3. One clone contained a 1250 bp insert and produced an insoluble β-galactosidase fusion protein in infected bacteria. The fusion protein was solubilized and was partially purified on DEAE cellulose. Competative ELISA experiments using the rabbit antiserum indicated the native and recombinant fusion protein contained similar epitopes. The fusion protein bound to agarose-phe. Upon its release it was shown to be capable of hemoglobin degradation. The sequence of the cDNA insert was determined. The predicted protein was larger than the native hemoglobinase indicating the enzyme may be synthesized as a precursor in the parasite. Gene titration experiments indicated the presence of approximately four copies of genes corresponding to this cDNA in the S. mansoni genome. Western blots of proteins obtained from life cycle stages indicated the rabbit antiserum recognized similar proteins in eggs, cercariae, schistosomula and adult worms. The extent of homology of these proteins with the adult hemoglobinase is under investigation.

**C 207** GROWTH AND DEVELOPMENT OF THIRD-STAGE LARVAE OF WUCHERERIA BANCROFTI IN CULTURE, E. D. Franke, W. Riberu and I. Wiady. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia.

Third stage larvae of Wuchereria bancrofti obtained from laboratory-infected mosquitoes grew and molted to the fourth stage (L4) in vitro. Best results were obtained in a cell-free culture medium containing NCTC 135 + Iscove's Modified Dulbecco's Medium (1:1; v/v) supplemented with 10% non-heat-inactivated human serum and an antibiotic/antimycotic mixture. Cultures were incubated at 37°C in an atmosphere of 5% or 8% CO2 in air. Molting was first observed 10 days after initiation of cultures, and was not synchronous. After 25 days of culture up to 100% of larvae were L4 with a mean length of 2.9 mm. On day 35 larvae averaged 4.6 mm in length. The culture system will provide an important tool for biochemical and immunological studies and screening potential anti-filarial compounds.

**C 208** CLONING OF AN ONCHOCERCA VOLVULUS SPECIFIC DNA SEQUENCE FOR USE IN SPECIATION OF L3's IN BLACKFLIES, William Harnett, Anne Chambers and R. Michael E. Parkhouse, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

The WHO has stated the need to differentiate the vector borne stages of O. volvulus from closely related parasites. One approach to this is to employ specific DNA probes. A genomic DNA library of O. volvulus has thus been prepared in the vector bacteriophage λGT10 and has been screened for specific DNA sequences by hybridisation with radiolabelled total DNA from several Onchocerca species. A clone has been isolated which interacts with DNA from O. volvulus but not O. gutturosa or O. gibsoni. This is currently being characterised and evaluated for use in endemic areas.

**C 209** Schistosoma mansoni MYOSIN PROTEIN: MOLECULAR AND IMMUNOLOGICAL CHARACTERISTICS. R. Harrison, G. Newport, J. McKerrow, P. Tarr, J. Kallestad and N. Agabian. University of California, Berkeley, Naval Biosciences Laboratory, 140 Warren Hall, Berkeley, CA 94720.

Our approach to isolating immunodominant S. mansoni antigens for subsequent functional identity studies has been to screen λgt11 cDNA libraries, representative of the adult mRNA population, with pooled sera from infected hosts. One clone, λcSB31, isolated in this manner was amplified and the fusion peptide extracted and purified. Sera from mice immunized with the fusion peptide responded to a 200Kd antigen in Western Blot analysis of S. mansoni worm homogenate and react to muscle tissue of worm sections in an indirect immunofluorescent antibody assay. Nucleotide sequence analysis of λcSB31 DNA revealed that the encoded 625 amino acid peptide exhibited significant homology to sarcomeric myosin rod protein. Immunofluorescent studies indicate that myosin is not present on the surface of schistosomula while Western Blot analysis shows that myosin is recognized by sera from infected humans, mice, rats and baboons suggesting that its immunogenicity is derived from degenerating worms. This interpretation was reinforced by the observation that sera from infected humans show a markedly stronger anti-myosin response after chemotherapy. The molecular arrangement of the schistosome myosin gene differs from that of evolutionary more advanced species in that schistosome myosin is derived from a single copy gene encoding a single mRNA transcript. The predicted structure of the protein, by analysis of the sequential arrangement of hydrophobic and hydrophilic amino acids, is consistent with the function of myosin protein studied in other species.

**C 210** PURIFICATION AND AMINO ACID SEQUENCING OF SCHISTOSOMA MANSONI SURFACE ANTIGENS, Charles Kelly,Matty Knight,Andrew Simpson,Michael Geisow and S.Ronald Smithers, National Institute for Medical Research,London NW7 1AA.

Surface antigens of schistosomula of Schistosoma mansoni can act as targets for protective immune responses.Two such antigens of $M_r$ 20K and 32K are also present in the adult worm and have been purified from the tegumental membranes.Polyclonal antisera raised against these antigens are being used to screen a cDNA library in the expression vector λgt 11.Additionally partial amino acid sequence data have been obtained and,based on sequence from the 20K antigen,three oligonucleotide probes have been synthesized.These have been used to screen a further cDNA library in λgt 10.Progress in cloning and sequencing these antigens will be presented.

Tryptic peptides derived from the purified antigens were screened for antigenicity and a thirteen residue peptide from the 20K antigen was shown to be recognised by human infection sera.This peptide has been synthesized and shown to correspond to a schistosomulum surface epitope.

**C 211** LONING AND CHARACTERIZATION OF DIROFILARIA IMMITIS ANTIGENS, Larry A. McReynolds†, Andres G. Grandea III†, Nur Asikin††, Martha Spiegelman#, Smith College, Northampton#, MA. New England Biolabs, Beverly, MA.†School of Medicine, University of Indonesia, Jakarta, Indonesia‡

A lambda gt 11 cDNA library of 400,000 recombinants was constructed from mRNA of adult female parasites. The recombinants were screened with antisera raised against adult female worms in rabbits. Three of the cloned recombinants was found to be antigenically cross-reactive with antisera raised against adult and L3s of D. immitis and adults of B. malayi. Antisera enriched for the cloned antigen recognized proteins of greater than 180,000 kD on a Western blot of adult D. immitis extracts. Sequence of the DNA was obtained by subcloning into M13, the open reading frame was used to determine a proposed protein sequence. A computer search of a protein data base found a sequence homology to the heavy chain of myosin from C. elegans. There was a 45% homology at the protein level and a 47% homology between the two DNA sequences. Most of amino acids that did not match between the two sequences were conservative amino acid substitutions. The localization of the presumptive myosin protein is being determined by light microscopy of different stages of the parasite life cycle. Two other phage recombinants were detected with anti-tubulin antisera. DNA sequence of the insert showed a high degree (82%) of homology at the amino acid level between D. immitis tubulin and the alpha tubulin of chicken. There was a 67% homology at the DNA level. This demonstrates that the tubulin sequence is highly conserved acrosse a wide evolution distance in contrast to myosin which is only moderately conserved between two helminths.

**C 212** MOLECULAR CLONING OF S. MANSONI ALLERGENS, Raymond J. Pierce, Jean-Marc Balloul, Brigitte Facon, François Trottein and André Capron, Centre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, Lille, France.

The clear involvement of the IgE response in immunity to reinfection in rat schistosomiasis and the demonstration of the same IgE-dependent in vitro cytotoxic mechanisms with sera from infected humans indicates that the cloning of S. mansoni allergens should be an objective in vaccine development. We constructed a cDNA library using S. mansoni adult worm mRNA in lambda $gt_{11}$ that was first screened with anti-S. mansoni rabbit serum, giving an expression frequency of 0.03 %. Screening of positive clones with sera from infected rats detected three clones that were recognized by both IgG and IgE antibodies. All three reacted more strongly with late than with early infection sera. One of the clones ($\lambda$ 3.13) fixed IgG antibodies that recognized a 105 kDa adult worm antigen. This clone contained a 800 bp insert and produced a 141 kDa fusion protein that was recognized by IgE antibodies in both rat and human infection sera. Work is in progress to determine the possible role of this antigen in IgE-dependant protective mechanisms.

**C 213** MOLECULAR CHARACTERISATION OF THE HYDATID ORGANISM, ECHINOCOCCUS GRANULOSUS, Arun K. Rishi, Carmen C. Cuesta and Donald P. McManus, Department of Biology, Imperial College, London, SW7 2BB, U.K.

A size-selected (0.5-5.0 kbp) genomic DNA library has been constructed in the bacterial plasmid pAT 153 using DNA extracted from a human isolate (Kenya origin) of the hydatid organism, Echinococcus granulosus. The library was screened using a panel of $^{32}$P-labelled total DNAs from various taeniid cestodes, including E. multilocularis and several characterised isolates of E. granulosus. Two recombinant plasmids with Echinococcus-specific inserts and a single recombinant plasmid (coded pEG 18) with a DNA insert unique for E. granulosus were identified. These recombinant plasmids with E. granulosus DNA inserts have now been used in restriction endonuclease, Southern transfer and hybridisation analysis to "Finger Print" E. granulosus isolates from different geographical regions including the hyper-endemic areas of Turkana, North-West Kenya and the Mediterranean littoral. The hybridisation patterns suggest that there are distinct intra-specific variants of E. granulosus of horse (donkey)/dog, camel/goat/dog, sheep/cattle/human/goat/dog and possibly pig/dog origin. This is not to say that the camel/dog, horse (donkey)/dog and pig/dog variants cannot infect man but that, to date, all human material examined (= protoscoleces, obtained from fertile cysts at operation) by us from different areas conforms by DNA analysis to the characteristics of the sheep/cattle/goat/dog form. In addition to the strain characterisation studies, the feasibility of using the cloned DNA fragment of pEG 18 - the recombinant plasmid with species-specific insert of approximately 2.3 kbp and moderately repetitive nature (copy number approximately 26) - as the basis of a simple field test for distinguishing eggs of E. granulosus from those of other taeniid cestodes, is currently being assessed.

**C 214** ANALYSIS OF THE STRUCTURE AND DIVERSITY OF THE SCHISTOSOME GENE ENCODING A MAJOR FEMALE SPECIFIC POLYPEPTIDE

Vanderlei Rodriguez, Maliha Chaudhri, Tina Walker, Matty Knight, Charles Kelly, Andrew Simpson.
National Institute for Medical Research, Mill Hill, London NW7 1AA.

The female schistosome continuously produces large numbers of eggs, a process which is central to the continuation of the life cycle and progression of disease. The cloning and characterization of the genes encoding the major egg components is thus an important facet of schistosome molecular biology. Analysis of S. mansoni cDNA libraries by differential screening has demonstrated a large number of apparently homologous clones encoding a polypeptide expressed only by egg producing females. The gene sequence suggests that it may encode an egg shell protein. Corresponding clones have been obtained from different strains of S. mansoni as well as S. haematobium. Sequence analysis and Southern blotting experiments are being undertaken to assess the degree of intra and interspecific variation exhibited by the gene. In addtion, these studies give insight into important structural features of the protein. Genomic fragments encompassing the S. mansoni gene have been obtained and are being sequenced.

C 215 MSP GENOMIC ORGANIZATION IN FREE-LIVING AND PARASITIC NEMATODES, Alan L. Scott, Daniel Sussman, Jonathan Dirman, Wesley K. Tamashiro, Chamberlain Diala and Samuel Ward. Johns Hopkins University and Carnegie Institution of Washington, Baltimore MD, 21205.

The major protein found in nematode sperm is a unique product of spermatogenesis common to many species of nematodes. The major sperm proteins (MSP) from a free-living nematode, Caenorhabditis elegans, and a parasitic species, Ascaris lumbricoides show a great deal of homology on both the DNA and protein level. However, the genomic organization of the MSP genes in these two species differs considerably; C. elegans has about 30 germline MSP genes while A. lumbricoides has only 3. In our studies on the regulation of antigen expression in parasitic nematodes, we investigated the genomic organization of the MSP gene in a variety of free-living and parasitic nematode species to gain information on how MSP expression may be regulated during spermatogenesis. MSP DNAs, cloned from both parasitic (Onchocerca volvulus, A. lubricoides) and free-living (C. elegans) nematodes, were used as heterologuous probes in Southern blot anlysis of genomic DNA from 8 parasitic nematode species and 2 free-living species. The hybridization patterns showed that the free-living nematodes had an estimated 12 to 25 copies of the MSP gene. The parasitic species had hybridization patterns indicating that they have 1 to 3 MSP genes. The reason for the complex organization of the MSP genes in the free-living nematodes is not clear but may reflect an adaptation to accomodate the environmental and physiological demands of a free-living existance.

C 216 CHROMOSOME SIZE DNA MOLECULES OF BRUGIA MALAYI. Sim, B.K.L., Wirth, D.F. and Piessens, W.F. Harvard School of Public Health, Boston, MA 02115.

We describe the fractionation of chromosome size fragments from Brugia malayi microfilariae by pulsed othangonal field gel electrophoresis. Ethidium bromide staining patterns reveal 2 chromosome size classes. The smaller size class consist of 1 single ethidium bromide staining band at approximately 500-700 kilobases and the larger size class contains 4 distinct bands of megabase sizes. Southern analyses showed the location of ribosomal genes on the single small ethidium bromide stained chromosome size fragment as well as all the fragments of the larger size class. We also used as probes, highly repeated B. malayi specific sequences. These sequences were absent on the single small chromosome size fragment but were located on a fragment approximately 1000 kilobases, not visible on ethidium bromide stained gels, as well as on all 4 of the chromosome fragments of the larger size class. DNA was cleaved in situ with restriction endonucleases Not I and Sfi I to make possible the analysis of large chromosomes. The distinct differences obtained in restriction patterns allowed the identification of the human B. malayi from B. pahangi the animal filariid.

C 217 PARTIAL CHARACTERIZATION OF A FEMALE-SPECIFIC DNA RESTRICTION FRAGMENT LENGTH POLYMORPHISM IN SCHISTOSOMA MANSONI, Loretta D. Spotila, Philip T. LoVerde and David M. Rekosh, SUNY at Buffalo, Buffalo, New York 14214.

A repeated element of Schistosoma mansoni DNA present at 10,000 copies/haploid genome has been partially characterized. Copies of the repeated element are both dispersed and tandemly repeated in DNA from both male and female worms. In DNA from female worms only, some copies of the element are present in an arrangement which is refractory to restriction by EcoRI and several other endonucleases. Thus, on a Southern blot of male and female DNA cut with EcoRI, hybridization with the cloned repeated element detects a large molecular weight (> 40kb) band in DNA from females but not in DNA from males. The large molecular weight female-specific DNA fragment can, however, be restricted with TaqI, Sau3A, and HinfI. This difference has provided a means for determining the sex of cercariae shed from snails with monomiracidial infections.

The sequence of the repeated element is being determined. The DNA of S. haematobium and S. japonicum contains repeated elements related to the S. mansoni repeat, and the genomic arrangement is similar in all three species. Since the female of the genus Schistosoma harbors the dimorphic sex chromosome pair (i.e. XY or ZW), it is hypothesized that the large molecular weight female-specific fragment is present on the Y (or W) chromosome.

**C 218** DEVELOPMENT OF DNA PROBES TO IDENTIFY AND SPECIATE FILARIAL PARASITES. Steven A. Williams[1,2], Catherine B. Poole[2] and Larry A. McReynolds[1]. [1]New England Biolabs, Inc. Beverly, MA and [2]Dept. of Biological Sciences, Smith College, Northampton, MA 01063.
We have cloned and characterized members of a repeated DNA sequence family found in filarial parasites of the genus Brugia (McReynolds, L.A., DeSimone, S.M., and Williams, S.A. 1986. PNAS, 83, 797-801). We have designated this repeat family the Hha I family because it is cleaved by that restriction endonuclease in both B. malayi and B. pahangi. The Hha I repeats are 322 base pairs in length and are organized in tandem arrays. These short repeats exist in about 30,000 copies per haploid genome or about 10% of the total Brugia DNA. This repeat is not found in other genera of filarial parasites. Due to the high copy number of this repeat in Brugia, cloned copies of this repeat can serve as extremely sensitive probes for the detection of Brugia in blood samples or in mosquitoes. To investigate the potential of the Hha I repeated DNA sequence for use as a species-specific probe, 30 copies of the repeat were cloned and sequenced from B. malayi and B. pahangi. A comparison of the repeated sequences derived from these data reveals 93% homology between the two species except for one 60 base pair long region with only 69% homology. The DNA sequences in this short region of divergence were used from the synthesis of species-specific oligonucleotide probes. The 29 nucleotide long probe specific for B. malayi and the 21 nucleotide long probe specific for B. pahangi were each shown to be extremely sensitive and species-specific (each showed at least 500-fold species-specificity). Each probe can detect a single microfilaria in crude preparations of DNA from blood or a single L3 in crude DNA preparations from mosquitoes.

**C 219** CLONING AND CHARACTERIZATION OF A cDNA FROM THE FEMALE GENITAL COMPLEX OF THE LIVER FLUKE FASCIOLA HEPATICA, Mario Zurita, David Bieber, Gordon Ringold and Tag E. Mansour. Department of Pharmacology, Stanford University, Stanford CA 94305.
The isolation and nucleotide sequence of a cDNA clone whose RNA is abundant in the female genital complex of Fasciola hepatica is presented. The pattern of expression in different fluke tissues and at different stages of miracidium formation suggests that this gene is expressed in the F. hepatica vitelleria. The deduced amino acid composition of the proposed protein agrees well with the reported amino acid composition of the F. hepatica egg shell. It contains a high percent of charged amino acids and the amino terminus has the leader sequence of a secretion protein. The putative protein is rich in glycine, lysine and tyrosine residues. The clone has homology with DNA from other trematodes but is only expressed in organisms in which the egg development is similar to that of the F. hepatica egg. The results suggest that the protein is conserved in organisms in which the miracidium formation occurs in fresh water. Studies on the expression, regulation and organization of this gene are now in progress. (Supported by a grant from the MacArthur Foundation.)

**C 220** MOLECULAR STUDIES OF A 24 KD APICAL MEMBRANE PHOSPHOPROTEIN FROM SCHISTOMSOMA MANSONI, Steven R. Karcz and Ron B. Podesta, University of Western Ontario, London, Canada N6A 5B7.
Reversible protein phosphorylation is widely recognized as a major mechanism by which metabolic pathways and processes in biological systems are regulated. The intricate relationship between transmembrane signal transduction and protein phosphorylation suggests that events leading to changes in the physiology of cells and organisms involve membrane-associated phosphoproteins. In this light, we have developed an in vitro system to study the phsophorylation of membrane-associated polypeptides using the inner bilayer of Schistosoma mansoni as a model. We have demonstrated that isolated membranes possess endogenous protein kinase activity resulting in the phosphorylation of a limited set of polypeptides ranging in molecular weight from 20 kD to 160 kD. Amongst these products is a prominent labelled polypeptide of approximately 24 kD whose phosphorylation is increased in the presence of manganese ion and sodium vanadate. This polypeptide is immunoprecipitable by an anti-membrane antiserum and appears to be amongst the most immunogenic inner bilayer polypeptides as judged by Western blot analysis. In addition, the phosphorylation of the 24 kD polypeptide appears to be enhanced in the presence of 5-hydroxytryptamine, an ambient signal molecule known to influence such diverse physiological processes as carbohydrate metabolism and surface membrane phospholipid synthesis. We have also investigated the properties of this polypeptide by lectin affinity chromatography and phase partitioning in Triton X-114. Taken together, these data suggest that the 24 kD polypeptide is an immunogenic integral membrane glycoprotein which is also phosphorylated and hence may play an important regulatory role in the intact schistosome. A cDNA clone whose fusion protein shares antigenic determinants with the 24 kD polypeptide has been isolated from an adult S. mansoni gt11 cDNA expression library. Studies are in progress to further characterize the 24 kD polypeptide and to determine the DNA sequence of a full length cDNA clone.

## *Immunology and Therapy*

**C 300** ANALYSIS OF THE MOLTING OF DIROFILARIA IMMITIS THIRD-STAGE LARVAE, David Abraham, Meisen Mok and Robert Grieve, University of Wisconsin, Madison WI 53706. The molting of D. immitis third-stage larvae ($L_3$) in vitro has been shown to be dependent on the presence of fetal calf serum in the culture media. In the present study, it was demonstrated that bovine albumin, added to several types of media at concentrations of 10-30 mg/ml, also proved to be an effective culture supplement for the induction of larval molting. These results were obtained when cultures were maintained at 37°; if cultures were incubated at 27° no molting occurred regardless of the presence or absence of culture supplements. The larval cuticle was examined at the ultrastructural level during the molting process. No evidence of a fourth-stage ($L_4$) cuticle was evident in larvae recovered from mosquitoes. After 24 hours in culture, there was evidence of cuticle formation, and by 48 hours the $L_4$ cuticle was completely formed. Ecdysis from the $L_3$ cuticle occurred beginning at 72 hours. $L_3$ placed in unsupplemented media at 27° showed no evidence of synthesis of the $L_4$ cuticle. Larvae placed in unsupplemented media at 37° displayed some cuticle formation and complete formation of the $L_4$ cuticle was seen in larvae placed in supplemented media at 27°. No evidence of ecdysis was seen in these worms. Two factors have thus been identified which are required for $L_3$ to molt, albumin in the culture medium and a temperature of approximately 37°. Lacking one of these factors, while retaining the other, will allow some synthesis of new cuticle, but will not support the complete molting process. Elucidation of the molecular mechanisms underlying the molt may provide insight into immunologic and pharmacologic strategies for control of this crucial part of the life cycle.

**C 301** SCHISTASOMA Mansoni SURFACE CARBOHYDRATETS PLAY A MAJOR ROLE IN THE DETERMINATION OF ANTIGENICITY. PIRLANTA OMER ALI AND ANDREW J.G.SIMPSON.

National Institute for Medical Research, Mill Hill,London NW7 IAA.

Analysis of antibody bining to intact Schistosomula of Schistosoma mansoni

in conjucntion with chemical modification of surface epitopes has demonstrated that at least 90% of available epitopes are defined by carbohydrate structures sensitive to TFMS and Periodate . These epitopes are expressed on glycoproteins of Mr 200 K and 38K and are preferentially recognised during a normal infaction rathar than following vaccination with irradiated cercariae.Both protective and blocking monoclonal antibodies recoqnise these epitopes and as such they are likely to play a role in the development and modulation of immunity in infected animals. Their recequntion during human infection is under investigaion and it has been found that high levels of IgG antibody recoqnising these eptopes are present during recent (acute)but not chronic infection.

**C 302** A 28 kDa PROTEIN OF SCHISTOSOMA MANSONI PRODUCED BY GENETIC ENGINEERING METHODS PROTECTS AGAINST EXPERIMENTAL SCHISTOSOMIASIS, Jean-Marc Balloul, P. Sondermeijer*, J.M. Grzych, J.P. Lecoq* and A. Capron, Centre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, Lille, France and *Transgène S.A., Strasbourg, France. We have isolated from Schistosoma mansoni adult worms a particular antigen with a relative molecular mass (Mr) 28 000 (28 kDa) present on the surface of the schistosomulum, and in the in vitro translation products from larval or adult parasite RNA with the same molecular weight and isoelectric point. This particular antigen could induce a significant level of protection either in a semi-permissive host as rat or in a permissive host as Balb/C mice. This 28 kDa antigen is also present in the adult worms of two other species of schistosomes : S. japonicum and S. bovis. Starting from adult worm RNA, an expression bank, containing 1 x $10^8$ independent recombinants, was constructed. Positive recombinants were detected using either rat or rabbit monospecific antisera raised against the 28 kDa antigen. Three independent candidates were selected and characterized by sequencing inserts in M13 derivatives using the termination reaction.

**C 303** IMMUNOCHEMICAL ANALYSIS OF THE EPICUTICLE OF PARASITIC NEMATODES. B. Betschart, M. Glaser, R. Kiefer, W. Rudin and N. Weiss, Swiss Tropical Institute, 4051 Basel

Cuticles were purified from adult worms of Ascaris suum and infective third-stage larvae ($L_3$) of Dipetalonema viteae as already described for adults of D. viteae (Betschart et al. Z.Parasitenkunde (1985) 71:87-95). The cuticular extracts were analysed by SDS polyacrylamide gel electrophoresis. It was found that the isolated polypeptides had the same molecular weights as the radioactively labelled polypeptides isolated after iodiation of adult D. viteae. The iodination studies showed that the proteins with the highest specific activity, presumably the surface proteins, could not be solubilized; this correlated with light and electron microscopic observations which showed that the epicuticle was resistant to solubilization. The insoluble cuticle pellets from D. viteae (adult and $L_3$) and A. suum were used to immunize mice. The antibody production was analysed by the indirect immunofluorescent antibody test and by immunocytochemistry. Specific antibodies against the epicuticle of A. suum could be detected, but none directed against the epicuticle of D. viteae. This is thought to be due to the complex structure of the D. viteae cuticle; even after exhaustive extraction the final pellet contains, besides the epicuticle, proteins of the cortical and median zones which are highly immunogenic. Since the epicuticle of A. suum could be purified to homogeneity, specific antibodies against the epicuticle were obtained, which showed also some cross-reaction with the epicuticle of D. viteae.

**C 304** ANTIBODY DEPENDENT ACTION OF PRAZIQUANTEL AGAINST SCHISTOSOMA MANSONI, Paul J. Brindley and Alan Sher, Laboratory of Parasitic Diseases, NIAID, NIH, MD 20892

Praziquantel (PZQ) at 3 × 250 mg $kg^{-1}$ is only about 20% as effective in curing C3H/HeN mice depleted of B-cells of patent infections with S. mansoni, as compared to its efficacy in intact animals. B-cell depleted mice given PZQ were still infected 7 weeks after chemotherapy with similar numbers of healthy, paired schistosomes to schistosome infected control mice not given PZQ. The efficacy of PZQ in B-cell depleted mice was completely restored by passively transferring immune mouse serum from mice infected for 6 weeks (IMS) or IMS minus IgG into these animals, and partially restored with IgG from IMS. In contrast, PZQ was just as effective at curing immunologically intact mice whether these mice were also treated with IMS or not. IgM and IgG antibodies were detected by immunofluorescent antibody tests bound to the surface of adult worms recovered from intact mice as early as 2 days after treatment with PZQ. These results, which confirm and extend previous observations by Doenhoff and co-workers, indicate that the mechanism of action of PZQ against schistosomes involves a synergy between the drug and anti-worm antibodies probably targeted against surface antigens on the adult parasite. Thus the most important anti-schistosomal drug in current use appears to require the host immune response for its chemotherapeutic action.

**C 305** REGULATION OF PROTECTIVE IMMUNE MECHANISMS IN SCHISTOSOMIASIS BY IgM BLOCKING ANTIBODIES, Monique Capron, J. Khalife, A.E. Butterworth*, J.M. Grzych and A. Capron, Centre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, Lille, France and *Department of Pathology, Cambridge University, U.K. After the demonstration of blocking antibodies during rat experimental schistosomiasis, the existence of such factors was investigated in human schistosomiasis. The depletion, in sera from S. mansoni infected patients, of a given isotype (IgM) either by protein A absorption or by fast protein liquid chromatography induced a significant increase in IgG-mediated killing of S. mansoni schistosomula by human eosinophils. Inhibition experiments showed that IgM-enriched fractions were able to inhibit eosinophil-dependent cytotoxicity mediated by IgG fractions. Both IgG and IgM antibodies from infected human sera immunoprecipitated antigens ranging between 30-40,000 apparent M.W., in the labeled detergent extracts of schistosomulum surface. The specificity of IgG and IgM for the 38,000 antigen was suggested by competition experiments using two radiolabeled monoclonal antibodies (IPLSm1, IPLSm3) directed against this antigen. The in vivo relevance of such IgM blocking antibodies in the context of human immunity to schistosomiasis was evaluated in two groups of children classified as resistant or susceptible to post-treatment reinfection. The mean levels of IgM antibodies were significantly higher in the susceptible than in the resistant group both before and after treatment. These results are consistent with the fact that immunity to schistosomiasis could be attributable not only to the existence of antibodies with defined effector function but also to the absence of blocking antibodies. They should also be taken into account in future vaccine strategies.

**C 306** A DEVELOPMENTALLY REGULATED SURFACE EPITOPE EXPRESSED BY THE INFECTIVE LARVAE OF *B.malayi* WHICH IS RAPIDLY LOST AFTER INFECTION,
[1]C.K.S. Carlow, [2]A.Spielman and [1]Mario Philipp, [1]New England Biolabs, Beverly, MA 01915, [2]Harvard School of Public Health, Boston, MA 02115.

*B.malayi* infective larvae express a species-specific surface epitope as demonstrated by immunofluorescence using a monoclonal antibody. The expression of the epitope is developmentally regulated, as it was acquired by the parasite during the L2-L3 ecdysis within the mosquito vector, but not before this time. Following injection into the mammalian host, infective larvae rapidly lost the ability to bind antibody. Within 24 hours of infection partial antibody binding occurred, which was visualized as a non-uniform ('patchy') fluorescent staining pattern. By days 3-5 (before the third moult), the epitope was no longer detectable. The loss of surface antigenicity by the parasite was not due to the acquisition of host albumin or immunoglobulin but may be caused by interaction with host cells. Larvae maintained *in vitro* in a cell-free culture at 37 °C for 4 days, or *in vivo* inside microdiffusion chambers which exclude cells (0.3 $\mu$m pore size) retained their antibody-binding capacity, whereas larvae from chambers that allowed cellular infiltration (3.0 $\mu$m pore size) showed 'patchy' fluorescence. Infective larvae dissected from mosquitoes and incubated in antibody at 37 °C also bound less antibody. These data suggest that two independent mechanisms may be involved in the disappearance of a surface epitope of *B.malayi* infective larvae following infection. Such processes may play an important role in immune-evasion *in vivo*.

**C 307** CHARACTERIZATION OF VACCINATION INDUCED ANTIBODIES IN THE BABOON SCHISTOSOMA MANSONI SYSTEM, Neil L. Cowen, Barbara J. Artelt and Raymond T. Damian, University of Georgia, Athens, GA, 30602.

Baboons (Papio Cynoceophalus) were vaccinated with live attenuated schistosomula and sera were collected fortnightly until termination at either 8 or 20 weeks post challenge. These sera were used to identify antigens recognized by vaccinated challenged animals but not challenged only animals. Western blots with soluble egg antigen (SEA) and detergent extracted worms (DEW) determined the time course of antibody production by isotype in sera from individual baboons. Most of the antigens uniquely recognized by the vaccinated animals were also immunoprecipitated from extracts of in vitro labelled worms and in vitro translation products of worm RNA. These proteins were of relative molecular weights 15, 17, 19, 29, 39 KD. A cDNA library made from adult worm mRNA is being screened with antibodies eluted from portions of protein blots corresponding to the antigens of interest.
(Supported by a grant from the University of Georgia Program in Biological Resources and Biotechnology and by the NIH (AI 18906).

**C 308** IMMUNOCHEMICAL AND BIOCHEMICAL STUDIES ON THE 30kDa EPICUTICULAR ANTIGEN OF BRUGIA PAHANGI, Eileen Devaney, School of Tropical Medicine, Liverpool, England.

Adult Brugia pahangi labelled with $^{125}I$ via the Iodogen method show a restricted profile of polypeptides, the major labelled component having a molecular mass of 30kDa. The 30kDa protein is antigenic in the infected host and is a Con-A adherent glycoprotein. The epicuticular localization of the 30kDa protein has been confirmed by, 1) in situ immunoprecipitation, in which $^{125}I$ labelled worms are incubated with infected jird serum and then solubilized and immunoprecipitated, 2) pre-adsorption experiments, in which incubation of live worms with infected jird serum removes antibodies which react with the 30kDa protein. A polyclonal antiserum has been raised in rabbits against the SDS-denatured 30kDa protein excised from acrylamide gels and has been used to follow the incorporation of radio-labelled amino acids into the protein. These experiments have demonstrated that the 30kDa epicuticular protein is continuously synthesized in the adult parasite (3 months and 6 months old) and that small amounts of the protein are released into the medium of worms cultured in vitro. The 30kDa protein does not appear to be present on the surfce of the infective third stage larvae of B.pahangi labelled via the non-permeable methods of Iodogen or $^{125}$Iodosulphanilic acid. Immunoprecipitaion of $^{125}I$ labelled $L_3$ with the immune rabbit serum (IRS) has also failed to demonstrate a cross reactive component of a different molecular weight on the surface. Experiments carried out with metabolically labelled $L_3$ (incubated for 48 hrs at 37°C) demonstrate that a protein of $M_r$ 30kDa is precipitated by the IRS. These observation suggest that the 30kDa protein may be rapidly synthesized and transported to the worm surface on infection of the definitive host.

**C 309** MOLECULAR INTERACTIONS BETWEEN SCHISTOSOMA MANSONI AND ITS INTERMEDIATE HOST, BIOMPHALARIA GLABRATA. Colette Dissous, Claude Dissous *, Jean-Marie Grzych and André Capron, Centre d'Immunologie et de Biologie Parasitaire, and * Unité INSERM d'Oncologie Moléculaire, Institut Pasteur, Lille, France.

Previous studies have shown that susceptibility of snails to parasite infection must imply both a suitable environment by the host and parasite evasion of host attack. We report here that the fate of S. mansoni in B. glabrata could be dependent on snail stimulating factors. Crude snail extracts increase methionine incorporation in miracidia and evidence is presented that a 80 kDa glycoprotein representing less than 0.01 % of total snail proteins and uniformly distributed in the snail body, is responsible for the stimulus. This glycoprotein specifically acts on the miracidial stage of the parasite suggesting that it could facilitate the snail penetration and the development of the parasite in its host. Moreover, it has been proposed that common molecular structures could aim at an easier adaptation of the parasite to its host. We have also shown that a major oligosaccharide epitope of the 38 kDa S. mansoni surface antigen is present on a 90 kDa B. glabrata glycoprotein. The characterization of this epitope on newly-hatched miracidia ruled out the possibility of an adsorption of mollusc components on parasites and raised the question of the role of this antigenic community in host-parasite relationships.

**C 310** BIOCHEMICAL AND IMMUNOLOGICAL PROPERTIES OF THE SURFACE OF ONCHOCERCA CERVICALIS MICROFILARIAE. M. K. Edwards[1,2], E. R. James[3] and M. Philipp[1]. [1]New England Biolabs, Beverly MA 01915; [2]Haverford College, Haverford PA 19041; [3]Medical University of South Carolina, Charleston SC 29425.

We analyzed the antigenicity and stability of the surface of skin microfilariae (mf) of O. cervicalis, a horse parasite. These mf express antigens on their surface that are cross-reactive with the cattle parasite O. lienalis and with the human parasite O. volvulus. The surface of living O. cervicalis mf was radioiodinated and solubilized in octyl-glucoside. Electrophoresis of this material showed 7 bands, 5 of which were specifically precipitated by antisera from rabbits immunized with O. lienalis mf or O. volvulus adults and mf. In addition, antisera from mice immunized with O. cervicalis mf bound specifically to the surface of freeze-thawed O. lienalis and O. volvulus mf as detected by immunofluorescence. Using both radiolabeling and immunofluorescence techniques, we found that the surface of O. cervicalis mf is dynamic. Radioactive iodine, bound to the surface, is released from live mf in a temperature-dependent fashion. Similarly, live mf, labeled on ice with mouse anti-mf antisera and secondary FITC-GAM, showed uniform surface fluorescence. When these mf were incubated at 37°C, but not at 4°C, the fluorescent pattern changed with time. First, small non-fluorescent patches arose, followed by an increasingly wide belt devoid of fluorescence, and finally, no visible fluorescence. These changes in the microfilarial surface suggest potential mechanisms for immune evasion by filarial parasites.

**C 311** EFFECT OF PRAZIQUANTEL ON SWINE CYSTICERCOSIS. Ana Flisser, Dante González, Aline Aluja, Agustín Plancarte, Dolores Correa, Patricia Ostrosky, Regina Montero and Eduardo Rodriguez-del-Rosal. Instituto de Investigaciones Biomédicas, UNAM, México, D.F., 04510.

Humans and swine are hosts of Taenia solium larvae. Cysticercosis is frequent in developing countries. Praziquantel has been used successfully for treatment of human neurocysticercosis. In order to analyze the effect of praziquantel, 50mg/Kg/day for 15 days were administered per os to 15 pigs with naturally acquired cysticercosis and to 5 normal pigs. Anticysticercus antibodies were looked for by ELISA and immunoelectrophoresis; chromosomic aberrations were searched in peripheral blood lymphocytes. After necropsy cysticerci were obtained from muscles and brain, viability was defined by oxygen consumption and evagination. Histopathologic studies were done on host-parasite interfaces. The humoral immune response increased after treatment regarding the intensity of serum antibodies and the number of antigens recognized. A genotoxic effect of praziquantel was found. A few days after treatment, muscle parasites were not viable, and the inflammatory reaction around them increased significantly. After 60 days, no recognizable cysts were found. In contrast, in the brain, oxygen consumption and evagination also decreased but cysticerci were not destroyed and the inflammatory reaction was small. The results clearly indicate that praziquantel killed the cysticerci. On the other hand, suggest that living parasites can balance the host immune response in such a way that, although recognized, they are not destroyed. When parasites die, the inflammatory reaction destroys and reabsorbs them, this process taking a longer time in brain than in muscle.

**C 312** CHANGES IN THE ORGANISATION OF THE SURFACE MEMBRANE UPON TRANSFORMATION OF CERCARIAE TO SCHISTOSOMULA OF *SCHISTOSOMA MANSONI*.

Michael Foley*, John R. Kusel[1], Peter B. Garland*. *Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland. U.K. [1] Department of Biochemistry, University of Glasgow G12 8QQ, Scotland U.K.

We have studied the early membrane changes involved in the transformation of cercariae to schistosomula of *Schistosoma mansoni* by two independent fluorescence methods: the differential binding of Merocyanin 540 (Mc540) and the technique of fluorescence photobleaching recovery (FPR). The cercarial membrane did not fluoresce on labelling with Mc540 whereas the outer membrane of newly transformed schistosomula did. Since Mc540 is considered to bind to fluid but not gel-phase bilayers, we inferred that transformation of cercariae to schistosomula is accompanied by conversion of the outer membrane from a gel-phase to a liquid-crystalline phase. In keeping with this conclusion we have found that the lateral diffusion of the incorporated fluorescent lipid analogue (5-N-(octadecanoyl)-aminofluorescein) as measured by fluorescence photobleaching recovery is greatly restricted in the surface membrane of cercariae but not schistosomula. Measurement of Mc540 fluorescence provides a convenient method of following the transformation of cercariae to schistosomula. These studies suggest a major reorganisation of membrane lipids in response to the transition by the worms to a parasitic existence in the host bloodstream and that only after such membrane changes occur can the parasite take up glycolipid molecules of mammalian origin.

**C 313** A MONOCLONAL ANTIBODY RECOGNIZES A MICROFILARIAL DETERMINANT CRITICAL TO PARASITE-VECTOR INTERACTIONS, Juliet A. Fuhrman, Sandy Urioste, Andrew Spielman and Willy F. Piessens, Harvard School of Public Health, Boston MA 02115.

The monoclonal antibody MF1 reacts with a developmentally regulated determinant on the surface of Brugia malayi microfilariae which is virtually absent on 2 day old microfilariae and progressively appears on worms that have resided in the infected host for longer periods. The ability of microfilariae to infect mosquito vectors likewise is an age-dependent process. Microfilariae less than 3 days old are unable to penetrate the mosquito midgut and hence do not develop into infective larvae. Because of the temporal coincidence of the ability to infect and the appearance of the MF1 determinant, we tested the possibility that the MF1 monoclonal antibody recognizes a parasite molecule critical to the process of midgut penetration. Microfilariae were mixed with the MF1 or an irrelevant control antibody and fed to permissive Aedes aegypti mosquitoes. Three hours after feeding, mosquito midguts were isolated, and the percentage of ingested microfilariae that had penetrated the midgut wall was determined. Pretreatment of microfilariae with MF1 antibody significantly decreased the percentage of worms penetrating the mosquito midgut wall by 22 to 96% compared to controls. This inhibition occurred in the absence of host-derived immune cells and when F(ab')2 fragments of the MF1 antibody were used. These results suggest that the MF1 antibody binds to and blocks a parasite component that is critical to the microfilaria's ability to interact with and penetrate the vector's midgut. These experiments demonstrate the potential of monoclonal antibodies for elucidating the biochemical basis of parasite-vector interactions.

**C 314** FLUORESCENT PROBES AND BIOPHYSICAL STUDIES OF THE SURFACE OF SCHISTOSOMA MANSONI

Peter B. Garland,* Michael Foley* and John R. Kusel‡
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Concomitant immunity, the phenomenon wherein chronically infecting S. mansoni elicit an immune response to which they themselves are resistant whereas newly invading Schistosomula are not, lacks an agreed molecular basis. It seems most likely that the structure and functional properties of the outer surface of parasites may contribute to concomitant immunity, but this membrane is difficult to study by conventional biochemical or biophysical means. To get around this difficulty we have made use of fluorescent probes allied with laser microscopy in order to ask specific questions about the outer surface. For example, it is possible to determine the freedom of incorporated membrane probes to diffuse laterally or rotationally, to gather information from environmentally sensitive probes, and to infer the distribution and proximities of probes or labelled membrane components either by direct vizualization with fluorescence microscopy or at the sub-microscopic level by Förster resonance energy transfer effects. These studies can be made more useful by extending them to the whole life cycle of the organism, and to simplified membranes such as those of body blebs. As a working hypothesis (Foley et al, 1986) we are using the idea that some property of the outer membrane, such as restricted lateral diffusion, limits the proper formation of complement membrane attack complexes or their analogues from cytolytic cells. Foley, M., MacGregor, A.N., Kusel, J.R., Garland, P.B., Downie, T. and Moore, I. (1986) J.Cell Biol. 103, 807-818

**C 315** INHIBITION OF IN VITRO LYMPHOCYTE PROLIFERATIVE RESPONSES OF OSTERTAGIA OSTERTAGI-SPECIFIC CELLS IN INFECTED AND IMMUNIZED CALVES, Louis C. Gasbarre, USDA, ARS, Animal Parasitology Institute, Helminthic Diseases Laboratory, Beltsville, MD 20705.

Economically, Ostertagia ostertagi (OO) is the most important cattle parasite in the U.S. Even though the parasite resides for a period of time in close proximity to tissues, very weak protective immunity is elicited. Significant interaction between parasite and immune cells is evident because within 3 weeks of infection the regional lymph nodes (LN) increase in weight 20-30 fold due to an increase in mononuclear cells. However, cells from these lymph nodes fail to respond to preparations of adult or larval worms or their metabolic products. But when the LN populations are examined for the frequency of OO-specific cells in a limiting dilution assay (LDA) for cellular proliferation, high frequencies (between 1:3000-1:10,000) of parasite-specific cells can be demonstrated. A similar dichotomy of results between conventional and LDA cultures is seen in the draining LN of calves parenterally immunized with OO antigens. The bulk culture inhibition can be overcome if culture supernates (SN) of mitogen-stimulated bovine lymphoid cells are incorporated into the culture media. Although, the primed populations do respond to the SN itself, the response is markedly enhanced by the addition of the priming OO antigens. Similar OO-antigen enhancement is not seen in populations primed with an unrelated antigen. These results indicate that although successful priming of bovine lymphocytes by OO antigens occurs, there is a subsequent inhibition of cytokine-mediated expansion of these clones. Current work is focused on the identification of the factors regulating growth, and a delineation of the mechanisms involved.

**C 316** PROTECTIVE MONOCLONAL ANTIBODIES AGAINST FASCIOLA HEPATICA CROSS REACT WITH S. MANSONI AND PROTECT AGAINST CHALLENGE WITH CERCARIA, Carolyn S. Hicks and Barbara L. Doughty, Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Texas A & M University, College Station, Texas 77843.

Monoclonal antibodies were originally produced against a soluble crude F. hepatica antigen and selected for antigenic specificity with an ELISA and indirect immunofluorescence against both juvenile and adult tegumental antigens. Monoclonal antibodies A12, D4, E11 and H8 all react with adult and juvenile tegumental antigens and give a range of protection against F. hepatica challenge from 39 - 58%. Western blot analysis of F. hepatica antigens shows that these monoclonal antibodies recognize seventeen different molecular weight fractions ranging from 27KD - 200 KD. Cross reactivity with S. mansoni has been investigated with both ELISA assays against SEA, SWAP and glutaraldehyde fixed cercaria. Monoclonal antibodies A12, D4 and E11 show cross-reactivity against cercarial tegumental antigens, D4 also reacts with adult S. mansoni tegumental antigens. Passive transfer of pooled ascitic fluid ( A12, D4, E11 and H8 ) against a challenge infection with S. mansoni gave 72% protection. These data indicate the potential for a multi-valent vaccine effective against trematode infections. This research was supported by the Texas Agricultural Experiment Station.

**C 317** NOVEL TEGUMENT GLYCOSPHINGOLIPIDS OF SPIROMETRA, G.G. Holz, Jr., B.N. Singh, R.W. Walenga, D.H. Beach, and J.F. Mueller, Departments of Microbiology & Immunology, Pediatrics and Pharmacology, S.U.N.Y. Health Science Center at Syracuse, NY 13210.

Neutral glycosphingolipids of S. mansonoides adults and plerocercoid larvae metabolically labeled with [U-$^{14}$C]glucose were isolated by column chromatography, and separated and collected by TLC. Eight pairs of radioactive and orcinol-positive fractions so-obtained were degraded and their derivatized components analyzed by capillary GLC and GC/MS. One low polarity pair [55% of total] contained galactose, dihydrosphingosine and phytosphingosine, and palmitic, stearic and 2-hydroxystearic acids. Fast atom bombardment and field desorption mass-spectrometry and $^{1}$HNMR identified two monogalactosylceramides. One contained phytosphingosine and 2-hydroxystearic acid [85% of total fatty acids], the other dihydrosphingosine and 2-hydroxystearic acid [55%]. Both were sequestered in tegument membrane fractions obtained by Triton X-100 treatment of adults and larvae. Four pairs of intermediate polarity TLC fractions [40% of total] appeared to be di- and trihexosylceramides containing galactose and glucose, dihydrosphingosine and phytosphingosine, and very long chain, saturated and unsaturated fatty acids [$C_{26}$, $C_{28}$, $C_{30}$]. Two pairs of high polarity [5% of total] were polyhexosylceramides containing neutral and amino sugars, dihydrosphingosine and phytosphingosine, and very long chain fatty acids. The highly hydroxylated galactosylceramides are characteristic of eukaryotic cells exposed to environments antagonistic to plasma membrane stability. The oligohexosylceramides containing very long chain fatty acids are unprecedented. Their biosynthesis and functions may offer targets for chemotherapy and immunotherapy of cestode infections.

**C 318** A DEFINED VACCINE AGAINST SCHISTOSOMIASIS BASED ON CELL-MEDIATED IMMUNITY, Stephanie L. James, E.J. Pearce, D. Lanar and A. Sher, George Washington University Washington, D.C. 20037 and NIH, Bethesda, MD 20892. Immunization with crude schistosome antigenic preparations by the intradermal route preferentially induces T cell-mediated immunity and protects mice against challenge Schistosoma mansoni infection (James, J.Immunol. 134:1956,1985). Induction of resistance in this model correlates most closely with sensitization for antigen-specific production of macrophage-activating lymphokine, but not with production of antibodies to parasite surface antigens (James, J.Immunol. 136:3872,1986). Humoral response in mice immunized with crude antigen is directed against a single protein antigen of $M_r$ 97,000, termed Sm-97, that is present in cercariae, schistosomula and adult worms and has been identified as a muscle component (paramyosin). Sm-97, purified by affinity chromatography, has been shown to significantly protect mice against S. mansoni infection. Sm-97 is both antigenic and immunogenic for cell-mediated immune responses; T cells from mice immunized with either crude antigen or purified Sm-97 respond to Sm-97 in vitro in lymphocyte blastogenesis and lymphokine production assays. Induction of cellular immunity against Sm-97 appears to be critical to the establishment of vaccine-induced resistance, since mouse strains that produce circulating antibodies to Sm-97 but do not demonstrate antigen-specific cellular responses are not protected. This is the first defined vaccine against experimental schistosomiasis to be based on induction of cell-mediated immunity, and opens the way for a new generation of immunoprophylactic regimens.

**C 319** MOLECULAR CLONING AND EXPRESSION IN E. COLI OF S. HAEMATOBIUM ANTIGENS: IDENTIFICATION BY HUMAN AND VACCINATED BABOON SERA. Matty Knight, Paul Hagan, Quentin Bickle, Andrew Simpson, S. Ronald Smithers.

Antigen expressing clones of Schistosoma haematobium have been identified from an adult worm λgt11 cDNA expression library by antiserum from infected Gambians and vaccinated baboons. Previous results have shown that the surface antigens of S. haematobium are species-specific. Accordingly, immunoscreening of the identified clones using serum from S. mansoni vaccinated mice was performed to establish the species specificity of these clones. One clone (λSh9) proved to be S. haematobium specific. The molecular characterisation of this clone will be presented.

**C 320** ISOTYPE SPECIFIC ANTIBODY RESPONSES TO BRUGIA PAHANGI IN RATS Rachel Lawrence and David Denham, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.

Rats infected with Brugia pahangi were used as a model for human lymphatic filariasis. Isotype specific antibody responses (IgG1, IgG2a, IgG2b, IgG2c, IgM, IgA ) were assessed quantitatively by radioimmunoassay and qualitatively by Western blotting to adult worms, microfilariae and infective larvae throughout infection. A comparison was made between fully susceptible athymic (nude) rats, and relatively resistant PVG strain rats. Specific antibody titres were found to be unrelated to total serum immunoglobulin levels. Antibody to a triplet of approximately 65 to 75 kD was associated with the onset of patency, indicative of stage specificity of these antigenic components, which were also resolved by surface iodination of microfilariae. Quantitative and qualitative differences in antibody responses and isotype switching are discussed in relation to susceptibility to infection and parasite development.

**C 321** THE HOST IMMUNE RESPONSE TO STAGE SPECIFIC SCHISTOSOME PROTEASES, James H. McKerrow, Matthew Petitt, Lisa Toy, George Newport, and Richard Hedstrom, Department of Pathology, University of California, San Francisco, CA 94143 and University of California, Berkeley, Naval Biosciences Laboratory, CA 94720.
Two Schistosome enzymes expressed only during specific stages of the parasite life cycle are targets for a host immune response in humans, monkeys, and mice. The cercarial elastase has been shown by Northern blot analysis and in situ cDNA hybridization studies to be expressed only in the sporocyst stage, and the subsequent translated protein has been shown by monoclonal antibody localization to be stored in the acetabular glands and not produced again after parasites have gained access to the blood stream. In contrast, the adult hemoglobinase has been shown by Northern blot analysis to be expressed in developing schistosomula and adults, but not in subsequent stages. Using purified enzymes as antigen in ELISA, patients with Schistosomiasis (sera obtained from Michael Stek) can be distinguished from controls. Only patients with recent infections appear to respond to the cercarial enzyme. Treated patients show a marked increase in reactivity to the hemoglobinase within two months of therapy, but a subsequent decrease. There is cross reactivity amongst all three major species of Schistosomes. Studies in monkeys (sera provided by Shirley Maddison) and mice indicate that the response to the cercarial enzyme appears in the first few days after infection, peaks at 7-9 weeks, and then diminishes. The response to the hemoglobinase, in contrast, appears first at three weeks after infection rises quickly and remains elevated.

**C 322** THE PRODUCTION AND CHARACTERIZATION OF HUMAN MONOCLONAL ANTIBODIES FROM PATIENTS INFECTED WITH SCHISTOSOMA MANSONI, Alfredo Miranda de Goes, Giovanni Gazzinelli and Barbara L. Doughty, UFMG and Centro de Pesquisas "Rene Rachou", Fundacao Oswaldo Cruz, Belo Horizonte, Brazil and Department of Veterinary Microbiology, College of Veterinary Medicine, Texas A & M University, College Station, Texas 77843.

Monoclonal antibodies reactive against Schistosoma mansoni egg antigens (SEA) were produced using three different methodologies. Peripheral blood mononuclear cells were isolated on Ficoll-Hypaque density gradients and cultured for 24 - 48 hours with SEA. B lymphocytes were then purified on an anti-human $F(ab)'_2$ Ig column and then either expanded in culture with pokeweed mitogen and adherent monocytes or infected with EBV. B lymphocytes expanded with both SEA and pokeweed mitogen were then fused with the mouse myeloma cell line P3x63. Those B lymphocytes that were transformed with EBV were expanded and tested for monoclonal antibody production and specificity using an ELISA assay against SEA, SWAP and glutaraldehyde fixed cercaria. Selected EBV transformed cell lines were then fused with the mouse-human heteromyeloma cell line SHM-D33. We currently have eight different hybrid lines (P3X63), and fourteen EBV transformed cell lines that are reactive with S. mansoni antigens. These lines are being cloned and further characterized for antibody specificity, quantitative antibody production and chromosome stability. The production of human monoclonal antibodies against S. mansoni antigens will provide monospecific reagents for prophylactic treatments and immunoregulatory regimens useful in clinical schistosomiasis. These studies were supported by the Edna McConnell Clark Foundation and NIH grant #A121776-02.

**C 323** IMMUNE EFFECTOR MECHANISMS AGAINST POST-SKIN STAGE SCHISTOSOMULA OF SCHISTOSOMA MANSONI. Edward J. Pearce, Alan Sher and Stephanie L. James*.
NIH, Bethesda, MD and *George Washington University Medical School, Washington D.C.

Newly transformed (3h) schistosomula are readily killed in vitro, by lymphokine-activated peritoneal macrophages or the macrophage cell line IC-21 (80% mortality over 48h in vitro). By 1-2 days of development however, schistosomula have become totally refractory to macrophage-mediated cytotoxicity. This insusceptibility to immune attack is evident in 7 and 10 day old schistosomula recovered from the lungs and in 10 day and 4-6 week old liver stage parasites. However, between 2 and 2.5 weeks of development in vivo, juvenile schistosomes recovered from the liver were susceptible to activated macrophages (25-65%). This is the first demonstration of a physiologically relevant immune effector mechanism which could account for post-lung phase challenge infection attrition in immunized animals. The interaction of post-lung stage schistosomes with macrophages was antibody independent. Furthermore in a quantitative immunofluorescence assay, sera from chronically infected mice, or mice vaccinated with irradiated cercariae, or by intradermal inoculation with soluble schistosome antigens plus BCG failed to react with the surface of 2.5 week old parasites. Immune recognition of susceptible post-lung stage schistosomula through antibody binding to surface antigens would therefore appear unlikely. However, immune sera did recognize excretory/secretory antigens synthesized by 2.5 week old parasites. Such antigens could participate in protective immunity via immune complex formation and/or activation of specific T cells.

**C 324** ROLE OF IMMUNE REACTIONS TO STRUCTURAL COMPONENTS OF O. VOLVULUS AND HUMAN EXTRA CELLULAR MATRIX COMPONENTS. Izaskun Petralanda[1,2], Luis Yarzabal[2], Willy Piessens[1]. [1]Harvard School of Public Health, Boston MA 02115; CAICET, Puerto Ayacucho, Venezuela.

The chronic manifestations of onchocerciasis include ocular lesions and destruction of certain dermal structures such as interlacing strands of collagen and elastic fibers. Little is known about the pathogenesis of these lesions. We previously described onchocercal enzymes which act on components of the host's extracellular matrix (EM) (collagen and elastin), and are immunogenic in natural and experimental hosts (1). We now report that structural components from O. volvulus appear to be collagenous in nature (by the criteria of SDS-PAGE and western blotting), are immunogenic for the human host, and are antigenically crossreactive (by the criteria of ELISA and western blotting) with vertebrate collagen and elastin. Simultaneously, we find that host antibodies to vertebrate EM components (collagen type IV and laminin, not fibronectin) are not totally absorbed by the collagenous components of the parasite. These findings suggest that two possible mechanisms are responsible for the progressive pathologic alterations seen in onchocerciasis. Parasite enzymes may be acting on host EM components, exposing new antigenic epitopes to the host's immune system and eliciting auto-immune responses. In addition, parasite structural antigens may elicit abs that crossreact with the host's own EM components.

(1) Petralanda, I., Yarzabal, L., Piessens, W.F. Mol. Biochem. Parasitol. 19(51-59). 1986.

**C 325** $^{31}$P NMR SPECTRUM OF THE RAT TAPEWORM DURING SHORT EXPOSURES TO BENZIMIDAZOLES, Edward P. Platzer, S. Nelson Thompson, and Robert W. K. Lee, University of California, Riverside, CA 92521.

The $^{31}$P NMR spectrum of the adult tapeworm, Hymenolepis diminuta, at 37°C during perfusion with saline contained 10 peaks. On the basis of chemical shifts and analysis of worm extracts, the phosphorus components included glucose-6-phosphate, fructose-6-phosphate, phosphorylcholine, glycerophosphoryl choline, glycerophosphoryl ethanolamine, nucleotide monophosphate,-diphosphate and -triphosphate, nicotinamide adenine dinucleotide and uridine diphosphate glucose. The mean concentration of nucleotide triphosphate was 0.86 nmol (mg fresh weight)$^{-1}$, the nucleotide triphosphate/ nucleotide-diphosphate ratio was 3.9, and the mean energy charge was 0.86. On the basis of nucleotide triphosphate concentration, the worms were viable for at least 3 h and the intracellular pH was maintained at approximately 6.7. Short-term perfusion with mebendazole at 11 or 27 μM in physiological saline containing 0.5% Tween 80 or 0.1% dimethyl sulphoxide had little effect on the nucleotide triphosphate level. Some cytological changes, however, were evident following perfusion of mebendazole. In contrast, exposure to 2,4-dinitrophenol caused a rapid decline in nucleotide triphosphate level. It was concluded that mebendazole does not exert its primary effect on oxidative phosphorylation.

**C 326** THE OCCURRENCE OF PHASE I DETOXICATION ENZYMES IN THE NEMATODE HELIGMOSOMOIDES POLYGYRUS AND PANAGRELLUS REDIVIVUS (NEMATODA) WENDY Y. PRECIOUS, UNIVERSITY COLLEGE OF WALES, ABERYSTWYTH, SY23 3DA.

A comparison was made between the occurrence of cytochrome P-450 and its associated enzymes 7-ethoxycoumarin O-deethylase, p-nitroanisole O-demethylase, and the aniline and biphenyl hydroxylases in the parasitic nematode Heligmosomoides polygyrus, its host the mouse Mus musculus and the free-living nematode Panagrellus redivivus. The occurrence of the Phase I reductase and hydrolase enzymes, azoreductase and N-deacetylase respectively were also investigated.

**C 327** Peroxidase activity in Heligmosomoides Polygyrus (Nematoda), Christine M. Preston and J. Barrett, UCW Aberystwyth, U.K.

The peroxidase enzyme of H. polygyrus was characterised, and determined to be functionally related to the cytochrome chain. The substrate specificity of the enzyme was determined, the order of activity for electron donors was dianisidine, pyrogallol, guaiacol, potassium ferricyanide, reduced cytochrome c. The enzyme was also active with the organic peroxides linoleic peroxide and cumene peroxide in addition to hydrogen peroxide. Lipoxygenase activity could not be demonstrated in H. polygyrus. The intracellular location of the peroxidase, using cell fractionation techniques in association with enzyme markers, revealed the peroxidase was associated almost exclusively with the inner mitochondrial membrane. Effects of inhibitors showed that the interaction of the peroxidase with the cytochrome chain was with the flavoprotein component of the chain. This is the region where the alternative chain, leading to cytochrome o branches. The peroxidase may, therefore, be involved in control at the branch point of the cytochrome chain in helminths.

**C 328** FUNCTIONAL ANALYSES OF THE EXCRETORY-SECRETORY MOLECULES OF TOXOCARA CANIS INFECTIVE LARVAE. Robertson BD, Rathaur S, McKerrow JH* and Maizels RM. Department of Pure & Applied Biology, Imperial College, London, UK. *Dept. of Pathology, School of Medicine, University of California, San Francisco, California 94143.

Toxocara canis infective-larvae are prolific producers of excretory-secretory (ES) antigens in vitro, which are currenly used in the diagnostic test, and have been extensively studied physicochemically. More recently studies have been directed towards elucidating the functional nature of these antigens, especially their role in parasitism and in the interplay between the host immune system and the parasite. Two categories of enzymatic activity have so far been established. Like a number of other parasitic helminths T.canis secretes acetylcholinesterase and this enzyme has been partially purified by affinity-chromatography using metal-chelating and Con A columns, and characterised with respect to molecular weight and substrate specificity. Similarly protease activity has also been found in the ES of the tissue-invasive larval stage. Studies with substrate SDS-PAGE gels, and an in vitro assay involving the degradation of an extracellular matrix has demonstrated serine protease activity associated with a number of components of radiolabelled ES.

**C 329** EXCRETORY/SECRETORY ANTIGENS OF ECHINOCOCCUS:GRANULOSUS: A SPECIES-SPECIFIC MOLECULE AND A CROSS-REACTIVE MOLECULE IDENTIFIED. James C. Shepherd and Donald P. McManus, Imperial College, London, UK.

The larval, or protoscolex, stage of the cestode E.granulosus excretes/secretes macromolecules into the hydatid cyst fluid which surrounds the parasite in the intermediate host. The cyst fluid is widely used as antigen for serological tests designed to detect circulating anti-parasite antibody in hydatid disease. All current tests give rise to false negatives, due to a low antibody response and immunocomplexing of antibody, and false positives, due to antigenic cross-reactivity. Using radioiodination, immunoprecipitation and SDS-PAGE a 12 KiloDalton subunit has been identified in cyst fluid of sheep, horse and human origin which is only bound by human antibodies to E.granulosus. This subunit may be species-specific. Human cyst fluid samples examined from different geographical regions all contain a similar if not identical subunit although not all hydatid patients produce antibodies to it. Monoclonal antibodies are being raised to this subunit and will be used to evaluate the feasibility of detecting it circulating in infected patients serum. A 61 KiloDalton antigen has been identified which is bound by human antibodies to a wide variety of infectious organisms and may be responsible for many false positives in serological tests. This extensive cross-reactivity may be due to the hapten phosphorylcholine, which is bound to the antigen, and is also present in a wide variety of other organisms.

**C 330** ANTIBODY RESPONSE OF VACCINATED GERBILS TO BRUGIA PAHANGI $L_3$ ANTIGENS. Wesley K. Tamashiro, Mohamed S. Ibrahim, and Alan L. Scott, The Johns Hopkins University, Baltimore, MD 21205.

Vaccination of gerbils with irradiated Brugia pahangi third stage larvae ($L_3$) has been shown to stimulate immunity to challenge infection through mechanisms which delay or inhibit patency and alter larval development resulting in lower adult worm loads. In our attempt to elucidate the mechanisms of immunity and to identify "protective" antigens, we compared the humoral response of vaccinated and unvaccinated gerbils to $L_3$ antigens. Vaccinated gerbils received 250 irradiated (20krad) $L_3$s subcutaneously followed 14 days later with a boost of 150 irradiated $L_3$s. Vaccinated animals were challenged 30 days post-boost with 100 normal $L_3$s and serum was taken at various times over an 80 day observation period. Unvaccinated controls which received a challenge infection alone became patent by day 60 whereas vaccinated animals remained amicrofilaremic. The post-challenge antibody response of unvaccinated animals to aqueous-soluble antigens was equal to or greater than the response mounted by vaccinated animals. However, the response to detergent-soluble antigens, as determined by Western blot analysis, was more pronounced in the vaccinated animals. Sera taken from vaccinated gerbils 5 days post-challenge recognized a broader spectrum of $L_3$ antigens than the unvaccinated controls. The humoral response of vaccinated and unvaccinated animals was studied further by immunoprecipitation of radiolabelled surface and excretory/secretory proteins. The different antigen recognition patterns displayed by immune and nonimmune animals has served to identify candidate proteins whose protective qualities will be tested in future vaccine trials.

**C 331** MECHANISM-BASED INACTIVATION OF GABA-TRANSFERASE: A RATIONAL OBJECTIVE FOR NEMATODE CHEMOTHERAPY, Stuart D.M. Watts and Adrian M. Atkins, The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, U.K.

Neurotransmitter function has proved to be a rewarding area in the development of anthelmintics. In particular, the excitatory, cholinergic system has been implicated as the target for several established anthelmintics as a result of retrospective mode of action studies. The inhibitory, GABAergic, system of nematodes has been relatively neglected although piperazine and, more recently, ivermectin have stimulated work on the GABA receptor.

An alternative strategy is to design specific inhibitors of the enzymes regulating GABA levels within the parasite, glutamate decarboxylase and 4-aminobutyrate aminotransferase (GABA-transferase, EC 2.6.1.19). Such a strategy has been extensively developed in studies of anti-convulsants acting on the mammalian central nervous system. We embarked on a programme of rational drug discovery with the objective of designing specific, mechanism-based inhibitors of nematode GABA-transferase in order to artificially boost levels of the endogenous inhibitory neurotransmitter, GABA. Three of these inhibitors, BW356U (a 3-pyrazolidinone), hydrazinopropionic acid and gabaculine respectively had dissociation constants of 16.2μM, 174nM and 395nM, with irreversible inactivation rate constants of $0.0087s^{-1}$, $0.045s^{-1}$ and $0.011s^{-1}$: they caused increases in GABA levels of 70%, 186% and 415% in exposed whole worms, in vitro.

**C 332** PARASITE ANTIGENEMIA IN BANCROFTIAN FILARIASIS DETECTED BY MONOCLONAL ANTIBODY-BASED ENZYME IMMUNOASSAY. Gary J. Weil, D.C. Jain, and S. Santhanam, Washington University School of Medicine, St. Louis MO 63110 and National Institute of Communicable Diseases, India

There is a need for improved diagnostic methods for filarial infections. We previously identified a 200 Kd parasite antigen in sera from patients with active Wuchereria bancrofti infections. Monoclonal antibodies that bind this antigen were used to develop a direct sandwich enzyme immunoassay. The assay was evaluated with sera collected in a W. bancrofti endemic area in South India. Filarial antigen was detected in sera from 56 of 57 microfilaremic patients, 9 of 64 amicrofilaremic patients with clinical filariasis, and 11 of 70 endemic controls. Antigen was not detected in nonendemic sera from patients with a variety of other filarial and nonfilarial helminth infections. Parasite antigen titers were significantly correlated with microfilaria counts in night blood smears ($r=0.64$, $p<0.01$). While the test performs best with serum (collected during the day or night), finger-prick blood samples dried onto filter paper can also be tested with only a slight decrease in sensitivity. The low sensitivity of the antigen assay in clinical filariasis patients may be caused by antibody competition. Antibodies to circulating W. bancrofti antigen were absent from all but 1 of 76 antigen-positive sera, but present in 41 of 65 antigen-negative sera from clinical filariasis patients. Despite this potential limitation, parasite antigen detection by enzyme immunoassay provides significant advantages over previously available methods for diagnosis of active W. bancrofti infection.