PARASITES: MOLECULAR BIOLOGY, DRUG AND VACCINE DESIGN

Organizers: Nina Agabian and Anthony Cerami

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Poster Sessions

Nonimmune Mechanisms of Parasite Killing-I

O 001 SURFACE THIOLS AND RESISTANCE OF *Entamoeba histolytica* TO COMPLEMENT AND AUTOLYSIS. Myriam Mogyoros and Carlos Gitler,Department of Membrane Research and Unit of Parasitology, Weizmann Institute, Rehovot,76100,Israel.
HM-1:IMSS trophozoites maintained for long periods in axenic growth, are highly susceptible to lysis by the human alternative pathway of complement (APC). These are pathogenic amoebae that have lost their complement resistance and their high virulence. When sufficient numbers are used to induce liver abscesses in hamsters, the liver passaged amoebae (P'HM-1:IMSS) shortly after isolation and growth in axenic medium, are resistant to complement lysis. Axenic growth of HM-1:IMSS trophozoites in the presence of active beef complement results in the gradual acquisition of complement resistance in the surviving amoebae (C'HM-1:IMSS). Both P'-HM-1:IMSS and C'-HM-1:IMSS trophozoites are highly virulent. Treatment of APC-labile HM-1:IMSS trophozoites with leupeptin, cysteine or DTT reduces APC-lysis to nearly zero. The amount of leupeptin required is higher than that which inhibits amoebal proteases. Treatment of trophozoites with DTNB or cystine activates an autolytic mechanism in HM-1:IMSS but not in C'-HM-1:IMSS. Complement resistance in all cases is associated with loss of lysis but with high consumption of complement.The thiol-containing proteins in the trophozoites surface have been identified by means of a new thiol-specific reagent Nα–Iodoacetyl-[^{125}I]-iodotyrosine (IAT).

O 002 CELL ATTACHMENT AND INVASION BY TACHYZOITES OF TOXOPLASMA GONDII.
Keith A. Joiner, Glaucia Furtado, Ira Mellman, Hynda Kleinman, Heini Miettinen, Lloyd H. Kasper, Lee Hall, and Steven A. Fuhrman. LPD, NIAID, and LDBA, NIDR, NIH, Bethesda,MD 20892, Dept of Cell Biology, Yale University, New Haven,CT, 06510 and Dartmouth Medical School, Hanover, NH 03756

Tachyzoites of T. gondii (T) attach to and actively invade nearly all eukaryotic cells, and reside within a parasitophorous vacuole which does not acidify or fuse with lysosomes. We are investigating the mechanisms for these events. T attachment to confluent monolayers of non-polarized J774 and CHO cells exceeded attachment to polarized MDCK cells by 5-6 fold, suggesting the possible involvement of basolateral extracellular matrix proteins . Laminin, but not fibronectin, enhanced T attachment to J774 cells, and the effect was blocked by the peptide YIGSR, which is directed to a cell attachment site in laminin. Surface iodinated T bands of 60/67kD and 14/16kD, the major components of the detergent insoluble residue from T, bound to laminin. The bands of 60/67kD were derived from a host cell receptor for laminin binding. These experiments suggest an important role for laminin and for parasite acquisition of host laminin binding molecules in cell attachment and entry by T.

We characterized the parasitophorous vacuoles (PV) of Chinese Hamster Ovary (CHO) cells infected with T. PV containing live T did not stain by immunofluorescence with the endocytic marker, lucifer yellow(LY), the acidification marker, acridine orange(AO), nor with antibodies to lysosomal glycoproteins (LGP). In contrast, phagocytosis of heat killed T by CHO resulted in LY+, AO+, and LGP+ vacuoles. These results suggest that PV manifest a generalized fusion block with all intracellular compartments. Although presensitization of T with antibody overcomes the fusion block in macrophages, the mechanism is unclear since ligation of Fc receptors (FcR) elicits a respiratory burst which may kill the parasite. To investigate the mechanism for this block, we presensitized T with antibodies to the parasite surface molecules p30 or p22, and allowed the parasites to infect CHO cells stably transfected with three isoforms of the murine FcRII receptor. Antibody presensitization increased the number of cell-associated T in dose related fashion, although anti-p30 was more effective than anti-p22 in mediating T internalization. PV were LY+, AO+, LGP+ and FcR+ in cells transfected with two phagocytosis-competent isoforms of FcRII, but not in cells bearing FcRII lacking a cytoplasmic tail or in wild type CHO cells lacking FcR. These experiments suggest that T form a fusion-incompetent PV, and that provision of a signal for phagocytosis or for phagosome-lysosome fusion overcomes this generalized fusion block and leads to parasite destruction.

O 003 IMMUNOMODULATION OF TH2 CELLS IN MURINE LEISHMANIASIS,
Richard M. Locksley, Michael D. Sadick, Frederick P. Heinzel, Bettie J. Holaday, and Robert L. Coffman, Department of Medicine, University of California San Francisco, San Francisco, CA 94143, and DNAX Research Institute, Palo Alto, CA 94304.

Fatal Leishmania major infection in BALB/c mice is characterized by expansion of CD4+ T cells and the appearance of IL-4 mRNA in lymphoid tissues together with marked elevation of serum IgE levels. These observations suggest that proliferation of Th2 cells accompanies progressive infection. In an attempt to interfere with Th2 expansion, BALB/c were infected with Leishmania major and treated with either recombinant IFNγ, neutralizing monoclonal anti-IL-4, or both. As compared to untreated controls, all 3 treatment groups had diminished IgE levels, although mice that received anti-IL-4 had the lowest levels. Mice that received recombinant IFNγ, despite lower IgE, developed progressive leishmaniasis indistinguishable from controls. Mice that received anti-IL-4 had significantly attenuated disease; some were completely cured and resistant to re-challenge. Mice that received a combination of IFNγ and anti-IL-4 were uniformly cured. These data support the hypothesis that Th2 cells are deleterious in murine leishmaniasis.

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Transcription and Processing

O 004 CONTROL OF VARIANT SURFACE GLYCOPROTEIN mRNA SYNTHESIS IN AFRICAN TRYPANOSOMES,
Piet Borst, Joost Zomerdijk, Marc Ouellette, Michèle Crozatier, Anneloor ten Asbroek and Janet Ampt. Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.
African trypanosomes, such as Trypanosoma brucei, evade the immune response of their mammalian host by repeatedly switching the composition of their surface coat, which mainly consists of a single protein species, the Variant-specific Surface Glycoprotein (VSG). There are some 10^3 different VSG genes per trypanosome. The actively transcribed VSG gene invariably resides near a chromosome telomere. Switching of the VSG gene transcribed can occur in two ways (reviewed in 1 and 2):
1. A new VSG gene is slotted into an active expression site (ES), displacing the old one.
2. An active VSG gene expression site is shut off and a silent one is activated. To determine how this is done, we have studied the structure of two expression sites (ES): the 221 site and the dominant ES (used for expression of chromosome-internal genes). We have cloned overlapping DNA segments corresponding to 65 kb of the 5′ flanking region of the VSG gene in the ES (3). By run-on experiments and UV-inactivation of transcription we have shown that the VSG 221 gene in the 221 ES is part of a single 60 kb transcription unit, containing several putative expression-site associated genes (ESAGs)(3,4). The dominant ES yields analogous results. In collaboration with Van der Ploeg (Columbia University, New York) we have recently shown that the putative promoter close to the VSG gene in the dominant ES (5) is fully dependent on the upstream promoter for its activity. We have now sequenced the promoter area of the 221 ES and are comparing the sequence of active and inactive variants of the promoter to determine whether sequence alterations are involved in the control of ES activity. We are also sequencing the ESAGs (6) and determining the cellular location of ESAG products. These experiments in progress should allow the delineation of the main factors involved in antigenic variation.

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5. C. Shea, M.G.S. Lee and L.H.T. van der Ploeg, Cell, 50 (1987) 603-612.
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O 005 RNA EDITING, Kenneth Stuart, Seattle Biomedical Research Institute, 4 Nickerson Street, Seattle, WA 98109

RNA editing is an RNA processing that occurs in the mitochondrion of kinetoplastid flagellates. It occurs by the addition and less frequently the removal of uridines from mRNA changing the sequence from that encoded in the gene. The 5' untranslated, coding sequence, 3' untranslated and poly(A) regions of mRNAs are edited but selectivly so since the sequence of these regions is not altered in all edited mRNAs. Editing creates initiation and termination codons and creates and extends translatable sequence thus modifying the functional capabilities of the mRNAs. The addition of the uridines within the poly(A) tails may alter the stability of the mRNA and thus its abundance. RNA editing appears to occur posttranscriptionally and to proceed in the 3' to 5' direction. The mechanism of RNA editing is uncertain but it appears to involve multiple steps involving polynucleotide regions. It probably involves the participation of a macromolecular complex provisionally termed the editosome. The editing activity is present in multiple life cycle stages but some transcripts are edited in a stage-specific fashion. This implies the existence of a regulatory system that controls the editing activity.

O 006 TRANSCRIPTION UNITS IN TRYPANOSOMES, Christian Tschudi* and Elisabetta Ullu*+, MacArthur Center for Molecular Parasitology, *Department of Internal Medicine and +Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

In African trypanosomes, protein coding genes are expressed as parts of polygenic and polycystronic transcripts. This mode of transcription is unique to these organisms and raises several questions concerning the location and structure of promoter sequences as well as how gene expression is regulated both quantitatively and qualitatively. Using the calmodulin genes as a model system, we have established a detailed transcription map covering the calmodulin gene cluster (containing 4 tandemly repeated coding regions) and approximately ten kilobases of upstream sequences. Two stable RNAs of 2 kb and 4 kb originate from the upstream region and are approximately 100-fold less abundant than mature calmodulin mRNAs. These RNAs are transcribed in the same direction as the calmodulin genes and the 3' end of the 4 kb RNA is located 120 nucleotides upstream from the spliced leader addition site of the first calmodulin gene. In addition, even lower abundance transcripts connect the 4 kb RNA and the calmodulin genes. The rate of transcription along the chromosome as well as the stability of the various transcripts has been evaluated and the results suggest the possibility that RNA processing events determine the output of stable RNAs from the calmodulin locus. The possibility that trans-splicing might take place co-transcriptionally is under investigation.

Molecular Evolution

O 007 RNA EDITING OF MITOCHONDRIAL TRANSCRIPTS IN LEISHMANIA TARENTOLAE - STUDIES ON THE MECHANISM, Larry Simpson, Janet Shaw, Norbert Bakalara, David Campbell and Agda M. Simpson, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024, and Department of Microbiology and Immunology, School of Medicine, University of California, Los Angeles, CA 90024

Editing of RNA transcripts in kinetoplastid protozoa involves the addition and also in some cases the deletion of uridine residues, and occurs for at least five mitochondrial genes (CYb, COIII, COII, MURF2, MURF3). The transcripts of several other structural genes are not edited (COI, ND1, ND4, ND5). The function of editing appears to be translational regulation. There are four basic types of editing: 1. The correction of internal frameshifts by addition of four or five uridines; 2. The modification of 5' ends of RNAs by addition and deletion of 20-39 uridines, creating more than 20 new N-terminal amino acids and methionine initiation codons; 3. The modification of the entire RNA molecule by multiple addition and deletion of uridines creating gene sequences in which more than 50% of the nucleotides are not encoded; 4. The apparent non-specifc addition of uridines to poly A tails and 3' untranslated regions. Both edited and unedited RNAs exist. A primer extension assay was used to show that the ratio of edited to unedited RNA varies from 0.89 for the L. tarentolae COII gene to 0.36 for the MURF3 gene (internal frameshift). Partially edited RNAs are not abundant enough to be detected by this method, thereby setting an upper limit of approximately 5% on partially edited transcripts for these genes. Edited RNA appears to be translated: antibodies generated against a peptide with the predicted COII carboxy terminal amino acid sequence reacted against a polypeptide of the expected size in Western blot analysis. Two mitochondrial-specific enzymatic activities have been detected which are possible candidates for editing enzymes: a terminal uridylyl transferase and an RNA ligase. The role of these enzymes in a posttranscriptional RNA editing scenario involving multiple endonucleolytic cleavage of a specific region, addition of multiple uridine residues, exonuclease cleavage and ligation, is being analyzed, as is the question of the nature of the information input required for the observed precise modifications of RNA transcripts.

Gene Expression in Development

O 009 GENE EXPRESSION IN TRYPANOSOMA CRUZI DEVELOPMENT, Samuel Goldenberg, Myrna C. Bonaldo, Marco A. Krieger & Monica A. C. Carreira. Dept. Bioquimica e Biologia Molecular, Fundação Oswaldo Cruz, Avenida Brasil 4365, 21040, Rio de Janeiro, RJ, Brasil.

The life cycle of T. cruzi involves three morphological and functional distinct forms and two intermediary hosts. The metacyclogenesis process occurs inside the invertebrate triatomine host and results in the transformation of the non-infective and proliferative epimastigotes into the infective metacyclic trypomastigotes. This transformation process has been studied in vitro under chemically defined conditions and the results indicate that epimastigotes have to adhere to a substrate prior to their differentiation. The analysis of the proteins expressed by adhered cells showed that polypeptides in the range from 48-52KDa are specifically synthesized by this population. In addition, the transformation of epimastigotes is triggered by cAMP, and important changes in the lipid composition of the parasites precede their morphological differentiation. The study of stage specific gene expression showed that adhered epimastigotes can express some trypomastigote antigens, indicating that the synthesis of these antigens precedes the morphological changes occuring during the metacyclogenesis process.

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O 010 SUBTELOMERIC RECOMBINATION AND REPETITIVE ANTIGEN GENES OF **PLASMODIUM FALCIPARUM**

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Macro-restriction mapping of **P.falciparum** chromosomes with enzymes that cut the AT-rich genome rarely has enabled us to generate physical maps of chromosomes 1, 2 and 5 from several clones. All chromosomes have a telomeric complex consisting of telomeric repeats, an ApaI site(s) and the repetitive element rep20. Deletions of rep20 can occur both in vitro and in field isolates, and these explain a major component of the observed size variation. Genes for internally repetitive antigens are clustered in the subtelomeric regions and subtelomeric deletions can abolish expression of a number of these, including all of the histidine rich protein genes and the RESA gene. The sequence of DNA across such a deleted RESA gene revealed that at least two deletion events, one involving non-homologous recombination, were required. A two-dimensional Pulsed Field Gradient gel system that can locate subtelomeric deletions on any of the 14 chromosomes has been developed and analysis of **P.falciparum** clones that differ in cytoadherence phenotype will be described.

Genes encoding internally repetitive antigens can show considerable sequence diversity in different isolates. Hence it appears that active recombination in the subtelomeric regions is responsible both for antigenic diversity and for chromosome size polymorphisms.

O 011 GLYCOSOMAL BIOGENESIS AND GLUCOSE TRANSPORT IN *TRYPANOSOMA BRUCEI*,

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African trypanosomes, as well as other kinetoplastid organisms, possess glycosomal microbody organelles which contain glycolytic enzymes plus several enzymes involved in other metabolic pathways. The constellation of enzymes contained in the glycosome varies during the life cycle. We have been concerned with how glycosomal proteins are targeted to the glycosome and how expression of genes encoding glycosomal proteins is regulated through the life cycle. Phosphoglycerate kinase (PGK) exists as both cytoplasmic and glycosomal isozymes that are stage regulated in a complementary fashion. Glycosomal PGK mRNA abundance is not the sole determinant of glycosomal PGK levels. Although blood forms from monomorphic and pleiomorphic strains of *T. brucei* possess similar glycosomal PGK activity, glycosomal PGK mRNA abundance is elevated in monomorphic strains. This suggests that, at least in part, translational control regulates the level of glycosomal PGK and that, at least in this case, monomorphic strains do not represent an accurate model for analysis of stage-regulated differentiation. In an effort to characterize glycosomal targetting signals, proteins synthesized in vitro from cloned mutant PGK genes were tested for their ability to specifically interact with glycosomes. Certain regions of the molecule could be deleted without eliminating targeting; however indications are that the targeting signals are complex. Analysis of chimeric cytoplasmic/glycosomal PGK proteins suggests that molecular topography may be important for the functional presentation of glycosomal targeting signals. A preliminary characterization of glucose transport across the cell membrane was performed. The trypanosome glucose transporter was not inhibited by several drugs which inhibit the human erythrocyte glucose transporter. Thus the trypanosome glucose transporter may make a suitable target for chemotherapeutic or prophylactic intervention.

Molecules in the Parasite Membrane

O 012 CYTOADHERENCE PROTEINS OF PLASMODIUM FALCIPARUM, J. Leech, C. Magowan, W. Wollish, C. Petersen, and R. Nelson. The Medical Service, San Francisco General Hospital, San Francisco, CA 94110. P. falciparum-infected erythrocytes (IRBCs) bind specifically to endothelial cells and some melanoma cells. Cytoadherence between IRBCs and venular endothelium permits the parasites to avoid the spleen and may cause cerebral malaria. Cytoadherence is prevented by isolate-specific antibody in immune serum and by incubating IRBCs with trypsin. In previous studies, a family of potential cytoadherence proteins was identified. The members of this protein family were large (Mr>200,000), radioiodinatable, isolate-specific, and trypsin-sensitive. We are characterizing this protein family and investigating its role in cytoadherence. We developed in vitro techniques for modulating the cytoadherence phenotype of cloned, knobbed parasites and observed that changes in cytoadherence were correlated with changes in the expression and molecular size of the radioiodinatable protein (RP). There were no changes in the expression of MESA-PfEMP 2 and KAHRP and one line of cytoadherent parasites lacked MESA entirely. Repeated selections of cloned parasites for the cytoadherent phenotype led to the expression of a multiplicity of RPs. IRBCs incubated with cyclohexamide remained cytoadherent and expressed the RP, but reexpression of both cytoadherence and the RP by trypsin-treated IRBCs was inhibited by cyclohexamide. Thus, both the cytoadherence ligand and the RP are turned over slowly, but parasite protein synthesis is required for their reexpression after trypsinization. The RP and its soluble proteolytic cleavage fragments comigrated on SDS gels with metabolically-labeled proteins, indicating that the RP is of parasite origin, and the RP was specifically immunoprecipitated by antibody in human immune sera, but not by polyclonal antibodies to human erythrocytes, band 3, glycophorin A, spectrin, or band 4.1. We conclude that the expression of the family of RPs is strongly correlated with the expression of the cytoadherence ligand and that these proteins are of parasite origin and are not altered host cell proteins.

O 013 MOLECULAR GENETICS OF THE MAJOR GLYCOPROTEIN AND REPETITIVE ANTIGENS OF *LEISHMANIA*. W. Robert McMaster*, Linda L. Button*, Anne E. Wallis*, Tom Frommel*, Yoshi Fujikura* John Webb* and Neil E. Reiner+, *Department of Medical Genetics and +Medicine, University of British Columbia, Vancouver, Canada V6T 1W5.

The major surface antigen of *Leishmania* promastigotes is a 63,000 MW glycoprotein referred to as Gp63 and is encoded by a family of direct tandemly linked gene copies which map to a single chromosome of approximately 700 KB. The tandem array of Gp63 genes is found in all *Leishmania* species examined although the total number of Gp63 genes varies amongst different species. For example in *L. major* there are five tandemly repeated Gp63 genes and a single gene copy linked as a direct repeat but separated from the tandem array of Gp63 genes. *L. donovani* contains at least eight Gp63 genes with at least six direct tandemly linked gene copies. DNA sequence analysis demonstrates that *L. major* and *L. donovani* Gp63 genes are highly conserved including a region encoding an Arg-Gly-Asp peptide shown to interact directly with the macrophage receptor for the complement component C3bi. Expression of the Gp63 gene locus is constitutive as Gp63 RNA and glycoprotein are present in both the promastigote and amastigote life stages of *Leishmania*. High levels of recombinant Gp63 from various *Leishmania* species have been synthesized in an *E. coli* expression system. Recombinant Gp63 conjugated to liposomes is currently being assessed as a possible vaccine to protect against cutaneous leishmaniasis.

Leishmania have a family of genes that encode proteins containing regions of tandemly repeated peptide sequence. Two *L. major* genes have been characterized that encode a highly conserved 14 amino acid repeat sequence and differ from each other in the 5' non-repeat sequence and the number of repeat units in that one gene encodes over 100 repeat units while the other encodes only 8. A homologous gene is present in *L. donovani* and the repeat sequence is identical to that encoded y the *L. major* genes. Antibodies against this 14 amino acid repetitive sequence detect a *L. major* protein of 240,000 MW. A third gene has also been characterized which contains over 100 repeat units encoding a 10 amino acid repeat sequence completely unrelated to the 14 amino acid sequence. Antibodies against synthetic peptides based on this 10 amino acid repetitive sequence react with a *Leishmania* promastigote cell surface protein of 205,000 MW. The role of these two different repetitive peptide sequences in immunity to *Leishmania* is currently being determined. The presence of regions of repetitive peptides is characteristic of many protozoan protein antigens, however, the *Leishmania* antigens are different from the characterized *Plasmodium* antigens in that the repeat sequences are highly conserved both within and between *Leishmania* species.

O 014 PROCYCLIN, A STAGE-SPECIFIC SURFACE ANTIGEN OF *T.BRUCEI*,
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Procyclin, an unusual glycoprotein consisting of a glutamic acid-proline dipeptide repeat for almost half its length, is expressed on the surface of procyclic trypanosomes. The genes encoding procyclin comprise a multigene family, some members of which are prone to rearrangement. We have previously shown that procyclin genes in cloned trypanosome strains from Kenya and Uganda show restriction fragment length polymorphisms. A detailed analysis of their genomic organisation has revealed that procyclin genes are arranged in tandem at 3 distinct loci (Pro A, B and C) in the Kenyan strain and that the polymorphism is due to a gene duplication in the Pro A locus.
A procyclin associated gene (PAG) has been identified upstream of the procyclin genes in the Pro A locus. PAG cDNA clones encode tandem repeats of 38 amino acids which show 50% homology to the repeat motif of the large microtubule-associated protein of *T. brucei* (Schneider et al., Science 1988, 241, 459). The highly repetitive nature of both procyclin and its flanking genes may explain the instability of certain loci.

O 015 THE LIPOPHOSPHOGLYCAN OF *LEISHMANIA DONOVANI*: STRUCTURE AND FUNCTION, S. J. Turco and T. B. McNeely, Department of Biochemistry, University of Kentucky Medical Center, Lexington, KY 40536.
The major cell surface glycoconjugate of *Leishmania donovani* promastigotes is lipophosphoglycan (LPG). Structurally, LPG is a polymer of repeating [PO_4→6Gal(β1,4)Manα1] units attached via a phosphosaccharide core to a novel *lyso*-alkylphosphatidylinositol anchor. A striking characteristic of the parasites is their ability to avoid destruction within phagolysosomes of host macrophages. We hypothesize that LPG plays an important protective role for the parasite by acting to modulate the production of the microbicidal oxidative burst. Consistent with this hypothesis are the results of three sets of experiments: (*i*) incubation of monocytes with a variant of *L. donovani* which lacks LPG resulted in the entry of the variant into the monocytes and its subsequent destruction, which was in contrast to wildtype *L. donovani*. (*ii*) addition of purified LPG to human peripheral monocytes attenuated the oxidative burst upon stimulation of the monocytes with either phorbal mystric acid or with opsinized zymosan. (*iii*) purified LPG from *L. donovani* was found to inhibit protein kinase C *in vitro*. Since protein kinase C is believed to be responsible for activation of the microbicidal oxidative burst of phagocytic cells, the potential *in vivo* consequence of this inhibition is that induction of oxidative burst by protein kinase C is precluded following infection. LPG was found to be a competitive inhibitor with respect to diolein, a noncompetitive inhibitor with respect to phosphatidylserine, and had no appreciable effect on protein kinase M and protein kinase A. Furthermore, the 1-O-alkylglycerol portion of LPG exhibited the most inhibitory activity toward the enzyme whereas the carbohydrate portion was not quite as effective. Taken together, these results indicate that LPG is critical in protecting the parasite within phagolysosomes of host. phagocytic cells

Molecular Modeling and Drug Design

O 016 MOLECULAR MECHANISMS OF DRUG RESISTANCE IN MALARIA, Alan F. Cowman, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.

The antifolate drug pyrimethamine is used as an effective antimalarial agent. However, naturally occuring drug resistance has been described for **Plasmodium falciparum** and **Plasmodium chabaudi**. A key residue has been identified that is involved in resistance to pyrimethamine of **P.falciparum** isolates from many different parts of the world. The possibility that this amino acid residue may be the major mechanism of pyrimethamine resistance throughout the world has important ramifications for the design of drugs effective against resistant parasites. Therefore it is of interest to know what effect other antifolate drugs, such as proguanil, have on these resistant parasites. Field data would suggest that pyrimethamine and proguanil resistance are not necessarily linked.

Pyrimethamine resistant lines of **P.chabaudi** can be easily selected on increasing doses of the drug. The mechanism(s) of resistance to this drug have been analysed in these lines and clearly show chromosomal rearrangements are involved.

O 017 UNIQUE PROPERTIES OF GLYCOSOMAL GLYCOLYTIC ENZYMES, Fred R. Opperdoes, Rik K. Wierenga, Wim G.J. Hol, Michèle Willson and Jacques Perié, International Institute of Cellular and Molecular Pathology, Brussels, Belgium; European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; State University of Groningen, Groningen, The Netherlands; Université Paul Sabatier, Toulouse, France

In the Trypanosomatidae, contrary to all other organisms, the glycolytic enzymes are located inside a microbody-like enzyme called "glycosome". Nine glycosomal enzymes and two of their cytosolic homologues have been purified to homogeneity from Trypanosoma brucei and compared with their homologues from other organisms. Three of the glycosomal enzymes have been crystallized and the three-dimensional structure of two of them has now been solved. The genes coding for five glycosomal genes and two cytosolic ones have been cloned and sequenced.

Together with the extensive data available on glycolytic enzymes from other organisms these results have allowed the identification of important molecular properties that uniquely differentiate glycosomal enzymes from their cytosolic homologues. For example, the existence of extra peptides, which are present as unique inserts or N-terminal or C-terminal extensions. These unique peptides are characterised also by the presence of the positively charged residues arginine and/or lysine. Where three-dimensional model-building has been possible (glyceraldehyde-phosphate dehydrogenase, phosphoglycerate kinase and triosephosphate isomerase) a characteristic pair of such clusters ("Hot spots") at 40 Å apart, has been recognized. Other structural differences have been identified, such as the subunit contact areas in glycosomal triosephosphate isomerase and near the NAD-binding region in glyceraldehyde-phosphate dehydrogenase.

Their physiological importance, unique location and their unique molecular characteristics makes these glycosomal enzymes the targets of choice for new chemotherapeutic approaches. A first attempt to synthesize inhibitors, specific for glycosomal enzymes has led to a series of compounds that specifically inhibit the glycosomal enzymes and leave their homologues from other organisms unharmed.

O 018 THYMIDYLATE SYNTHASE AND DIHYDROFOLATE REDUCTASE IN PARASITES, Daniel V. Santi, Departments of Biochemistry and Biophysics and Pharmaceutical Chemistry, University of California, San Francisco, CA 94143. Studies of the bifunctional thymidylate synthase (TS) - dihydrofolate reductase (DHFR) from protozoans and monofunctional counterparts in other organisms will be described. In particular, we will describe investigations of the bifunctional enzyme from *Leishmania*. These will be related to parallel studies on the structure, function and inhibition of related monofunctional counterparts from other sources which are well understood. Finally, we will describe studies which have led to the cloning and expression of TS and DHFR from the organism *Pneumocystis carinii*.

Vaccines and Vaccine Strategies

O 019 VACCINES AND VACCINATION STRATEGIES: HELMINTHS, Graham F. Mitchell, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

For many helminth infections for which safe, cheap and effective vaccines are desired, basic research has not yet resolved the issue of what immune effector mechanisms are responsible for host resistance and what immune evasion mechanisms are responsible for chronic parasitism. The development of "natural antigen" vaccines based on acquired resistance relies on the unequivocal identification of immunological correlates of resistance to infection or disease in exposed individuals or in relevant model systems. An alternative approach is to identify an "Achilles heel" in the parasite involving a functional molecule of poor natural immunogenicity. Hopefully, this "novel antigen" is relatively invariant in the interests of preserving function and from a lack of immune pressure to vary and is not a complete molecular mimic of a host molecule. The greatest encouragement to vaccine development comes from a clear demonstration of concomitant immunity. In its simplest form, this state of immunity involves elimination of establishing infective stages of a parasite in a challenged infected host. Encouragement also derives from bell-shaped age-prevalence curves and/or a sharp decline in intensity of infection with age despite continuing exposure. Examples will be given of prototype helminth vaccines based on antigens identified by five approaches - analysis of concomitant immunity, use of a functional monoclonal antibody (and perhaps T cell lines and clones), analysis of genetically-based resistance in model systems, isolation of a subset of molecules by fractionation of crude extracts that protects, and finally, analysis of acquired resistance to reinfection in human populations. The principal helminth-diseases covered in outline are schistosomiasis, trichinosis and cysticercosis.

O 020 CELL-MEDIATED VACCINES AGAINST SCHISTOSOMES AND LEISHMANIA, Alan Sher, Edward Pearce, Stephanie James and Phillip Scott, Immunology and Cell Biology Section, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD 20892 and Biomedical Research Institute, Rockville, MD 20852.

Cell-mediated effector mechanisms dependent on CD4+ T lymphocytes have been shown to play a major role in immunity against schistosomes and Leishmania. We have developed murine vaccine models against these organisms which employ non-living parasite extracts with the aim of identifying the relevant immunogens as well as investigating at the T cell/antigen level the manner in which protective cell-mediated responses are induced and regulated. The first model employs the intradermal injection of soluble schistosome (S. mansoni) extracts with an adjuvant, BCG, and the second the intraperitoneal injection of promastigote (L. major) extract (SLA) with C. parvum. In both models, protective immunity fails to develop in athymic mice and can be transferred to normal recipients with immune T cells but not with immune sera. In the Leishmania model T cell lines and clones belonging to the Th1 subset mediate the transfer of protection whereas transferred Th2 cells actually exacerbate infection. Our current hypothesis is that a similar dichotomy in Th subset function may occur in the schistosome model. Thus, strong IFN-γ responses are observed when T cells from vaccinated mice are stimulated with parasite antigen and in vivo treatment with anti-IFN-γ antibodies results in partial depletion of immunity. In contrast, cytokines produced by Th2 cells (e.g., IL-5) predominate in the response to schistosome infection in non-vaccinated hosts and treatment with anti-IL-5 antibodies fails to reduce vaccine immunity.

Two different approaches have been employed to identify protective vaccine molecules. In the schistosome model, the uniquely monospecific antibody response of vaccinated mice led to the identification of paramyosin, an internal myofibrillar protein as one protective molecule in the original vaccine preparation. The major epitopes in the molecule responsible for eliciting Th1 (i.e., IFN-γ) responses have now been mapped using synthetic peptides spanning the known sequence of the protein. In contrast, in the leishmania model, a candidate vaccine antigen of approximately 10,000 kD has been identified directly on the basis of its recognition by a protective Th1 clone in T cell immunoblotting experiments. We are currently attempting to determine whether the selective induction of protective, Th1 responses by these schistosome and Leishmania antigens is a function of their chemical structure or mode of presentation during vaccination or challenge.

Epidemiology and Field Study - Molecular Approaches, Model Systems

O 021 MOLECULAR DIAGNOSTICS AND EPIDEMIOLOGY, D.F. Wirth. Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115.

DNA probes have been developed for the diagnosis of several tropical diseases including malaria, leishmaniasis, filariasis, onchocerciasis, amoebiasis and tuberculosis. These probes were originally isolated as highly repeated DNA sequences present either in genomic or organelle DNA. Direct analysis of clinical samples using DNA probes has been successful for all of these diseases and the probes compare favorably with standard diagnostic methods. The DNA probe specific for P. falciparum has been developed into a field test and has now been used on 15,000 patient samples, most extensively in Thailand where it is currently being used in epidemiological studies. The DNA probes specific for Leishmania mexicana and Leishmania braziliensis have been used extensively for direct diagnosis of patient samples in Manaus, Brazil. Recent work using the Polymerase Chain Reaction (PCR) method has greatly increased the sensitivity of all the DNA probes which we have developed. Amplification of target DNA from direct clinical samples, frozen biopsies and embedded tissue has been possible for Leishmania mexicana and Leishmania braziliensis specific probes. Similarly, a million-fold amplification of P. falciparum specific sequences has been achieved in the laboratory.

The PCR method must now be field-adapted but opens the possibility of using DNA probes of greater specificity which are present in low copy number. Our efforts are focused on a DNA probe specific for drug-resistant P. falciparum. We have identified two P. falciparum genes, Pfmdr1 and Pfmdr2, related to mammalian P-glycoprotein genes and are currently investigating the role of this gene in drug resistance in P. falciparum. In multidrug resistant-mammalian tumor cells, resistance is associated with increased expression of the P-glycoprotein which is often the result of an amplification of the gene. We have preliminary evidence that Pfmdr2 is amplified in mefloquine-resistant P. falciparum.

Parasites: Molecular Biology, Drug and Vaccine Design

Parasite Killing Mechanisms; Transcription, Processing and Transformation

O 100 Ro-15-5458 SELECTIVELY INHIBITS THE TRANSLATION OF mRNA ISOLATED FROM ADULT SCHISTOSOME, Feleke Eshete and James L. Bennett, Department of Pharmacology, Michigan State University, E. Lansing, MI 48823. Ro 15-5458 is a potent, experimental antischistosomal drug developed by Hoffmann LaRoche. It is active against all geographic strains of Schistosoma, in various experimental animals, at 15.0 mg/kg. The drug has a slow onset of action i.e., parasites shift from the mesenteric veins to the liver 5 days after dosing. The drug has no in vitro antischistosomal activity. Parasites removed 3 days after dosing from infected mice have normal metabolic and physiological signs except for a significant reduction in parasite protein content which is associated with a significant reduction of the incorporation of labeled leucine and thymidine into TCA insoluble fractions. The translation of proteins from mRNA isolated, as early as 12 hours after dosing infected mice, was reduced by 50% relative to controls. This action appeared selective since translation of liver mRNA isolated from mice given multiple doses of the drug was not reduced. Kinetic studies suggest inhibition of translation is due to improper initiation of protein synthesis. Removal of the thiazolyazo group from the parent compound eliminates antischistosomal activity and the drug-induced lesion of parasite mRNA.

O 101 SINCE HYDROGEN PEROXIDE IS TOXIC TO MICROFLARIAE, HOW DO THEY SURVIVE *IN VIVO*?
Heather L. Callahan, Rosalie K. Crouch, and Eric R. James, Department of Ophthalmology, Medical University of South Carolina, Charleston, SC 29425.

Despite tissue eosinophilia and elevated immunoglobulin levels associated with onchocercal infections, mf usually remain apparently undamaged. Phagocytic cells enriched for eosinophils have been shown toxic for mf *in vitro*. However, it is not known whether this toxicity was mediated through the release of granule proteins or oxidants. Using *Onchocerca cervicalis* as a model for *O. volvulus*, we investigated the effect of the reactive oxygen species hydrogen peroxide, singlet oxygen, hydroxyl radical, and superoxide radical on mf *in vitro*.

Hydrogen peroxide and singlet oxygen are toxic for mf. Catalase, which scavenges hydrogen peroxide, protects in all systems where hydrogen peroxide is present. Inactivated catalase, superoxide dismutase (SOD), singlet oxygen scavengers and most hydroxyl radical scavengers, do not protect mf in any system. One hydroxyl radical scavenger, thiourea, does protect mf. However, protection appears to be the result of non-specific scavenging of hydrogen peroxide. Sensitivity to hydrogen peroxide toxicity correlates with the low levels of catalase and absent glutathione peroxidase found in mf.

Since levels of hydrogen peroxide comparable to those generated by eosinophils are toxic for mf *in vitro*, it is unclear why mf are not killed *in vivo*. Our preliminary results indicate that one explanation may be that exposure of mf to non-toxic levels of hydrogen peroxide induces resistance to previously toxic levels of hydrogen peroxide. We have also found that mf have relatively high levels of SOD and are currently determining if this enzyme has a role in the protection of mf using immuno-histochemical techniques. [Supported by NIH grants EY05757, EY06462 and EY07542].

O 102 ROLES OF TUMOR NECROSIS FACTOR AND OTHER CYTOKINES IN MALARIAL IMMUNOPATHOLOGY, Ian A. Clark, Geeta Chaudhri, Susan Ilschner and William B. Cowden, Department of Zoology and Experimental Pathology, Australian National University, Canberra, ACT 2601, Australia.

Evidence is accumulating that overproduction of immunomodulators of host origin, particularly tumor necrosis factor (TNF), may have central roles in the pathogenesis of illness and pathology of malaria and similar acute infections. For example, malaria infection primes for TNF release; ourselves and others have detected TNF in the serum of acutely ill patients and experimental animals; malarial antigens can trigger release of TNF from macrophages; and, at least in the mouse model of cerebral malaria, antibody to TNF prevents development of pathology. In our mouse model of malaria (*Plasmodium vinckei*) we have reproduced the terminal pathology seen in this disease, including dyserythropoiesis and fetal death, by injecting r TNF (Asahi). Since we have also detected IL-1 in the serum of some malarial patients, and since TNF and IL-1 are synergistic in certain other circumstances, we have investigated whether these two monokines are synergistic in producing the pathology seen in malaria. This proved to be the case, nanogram quantities of IL-1β (Merck) reducing TNF requirement about 10 fold. Furthermore, IFN-γ (which has also been detected in acute malarial serum) synergized in this context with both TNF and IL-1, alone or separately. Thus the pathology of this disease appears to be the result of a complex interaction involving at least these three cytokines, not TNF alone.

O 103 **ANALYSIS OF B- AND T-CELL RESPONSES TO THE REPETITIVE PF 11-1 ANTIGEN OF *PLASMODIUM FALCIPARUM*.** Philippe Dubois, Artur Scherf, Marika Pla[+] and Luiz Pereira da Silva, Institut Pasteur, 25-28, rue du Dr. Roux,75015, Paris, [+]U93 INSERM, Hopital St Louis, 2, Pl. du Dr Fournier, 75010 Paris, France

We isolated from a genomic expression library of the malaria parasite *P. falciparum* a clone coding for a highly repetitive antigen associated with the membrane of infected erythrocytes, Pf 11-1. The major part of this molecule consists of at least 200 degenerated repeats of nine amino-acids, whose consensus sequence, E-E-V-V-E-E-V-V-P, is highly homologous to repeats of other malaria antigens, Pf RESA and Pf 332, and to part of Thymosinα1. These repeats show a high degree of cross-reactivity, and secondary structure analysis predicts that they share a high tendency to form amphipathic alpha-helices.

The antibody and T-cell responses to the two most frequent types of repeats of Pf 11-1 were analysed in five different H-2 congenic mice strains using two synthetic peptides (P9A: Y-P-(E-E-V-V-E-E-V-V-P)$_2$-K; P9B: Y-P-(E-E-L-V-E-E-V-I-P)$_2$-K). The antibody response to both peptides is restricted to H-2^d and H-2^k haplotypes. Specific antibodies do not discriminate between the two peptides as assessed by ELISA. The MHC restriction was confirmed by analysing the T-cell response to both peptides. T-cell lines specific for each peptide were derived from H-2^d responding mice. Although the peptides differ in four positions of the hydrophobic parts of the helices, no significant differences were observed in the antigen presentation between P9A and P9B peptides. In contrast, no proliferative response was observed when the two T-cell lines were tested against the heterologous peptide, suggesting that the variable hydrophobic amino acids are involved in T-cell recognition. We are currently investigating: i, if cross reacting sequences from Pf RESA and Pf 332 antigens or self products such as Thymosinα1 lead to cross-activation of the Pf 11-1 specific T-cell clones; ii, if other variant repeats of Pf 11-1 antigen are restricted to different H-2 haplotypes and that the totality of the repeats may be subjected to no restriction.

O 104 SPOROZOITE VACCINE INDUCES GENETICALLY RESTRICTED T CELL ELIMINATION OF MALARIA FROM HEPATOCYTES. Stephen L. Hoffman, Daniel Isenbarger, Ana Szarfman, Gary W.Long, Martha Sedegah, David S. Finbloom and W. Ripley Ballou. Naval Medical Research Institute, Bethesda, Maryland, 20814, and Walter Reed Army Institute of Research, Washington, D.C. 20307

Mice immunized with irradiation attenuated malaria sporozoites are solidly protected against challenge by an immune response that has been shown to require CD8+ T cells . The target of this immunity has not been established. We report that immune BALB/c mice develop malaria specific, and CD8+ T cell-dependent inflammatory infiltrates in their livers after challenge with Plasmodium berghei sporozoites, and that spleen cells from immune BALB/c and C57BL/6 mice eliminate hepatocytes infected with the liver stage of P. berghei from *in vitro* culture. The activity against infected hepatocytes is not inhibited by anti-interferon γ and is not present in culture supernatants. It is genetically restricted, indicating that malaria antigens on the hepatocyte surface are recognized by immune T effector cells. Further subunit vaccine development will require identification of the antigens recognized by these T cells, and a method of immunization that induces such immunity.

O 105 ROLE OF CD4+ T CELLS AND SPLEEN IN IMMUNITY TO THE MURINE MALARIA, *PLASMODIUM VINCKEI*, Sanjai Kumar, Michael F. Good, Franklin Dontfraid, Joseph M. Vinetz, and Louis H. Miller, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD 20892

Infection of mice with *P. vinckei* is invariably fatal. However, mice develop solid immunity after two cycles of infection and drug cure. Here we present evidence that such immunity is mediated by CD4+ T cells through effector/inducer function, not antibody help. Passive transfer of antibody from immune mice into athymic nu/nu mice does not render the recipients immune. B cell-deficient C3H/HeN mice can develop immunity to *P. vinckei* reinfection without making parasite-specific antibodies. *In vivo* depletion of CD4+ T cells in immune BALB/c mice completely abrogates their immunity. This loss of immunity can be reversed by transfer of CD4+ T cells from spleens of immune mice into CD4-depleted immune mice. Interestingly, immune CD4+ T cells fail to transfer immunity into normal BALB/c or histocompatible BALB/c nude mice. Splenectomy of immune BALB/c mice reverses their immunity. Repletion of splenectomized mice with syngeneic immune spleen cells does not reconstitute immunity. These observations demonstrate the requirement of an architecturally intact malarial spleen as well as specific CD4+ T cells for expression of immunity to *P. vinckei*.

We further found that mice could not be immunized with *P. vinckei* antigens in various adjuvants. While such vaccine preparations may induce protective T cells, they may fail to produce the splenic architecture required for blood stage immunity. A vaccine based on cellular immunity against blood stage infections may require a combination of malarial antigens and adjuvants or live organisms such as salmonella or BCG to induce both protective T cells and proper splenic architecture.

O 106 ANTIGEN RECOGNITION BY ANIMALS IMMUNE TO *ONCHOCERCA* MICROFILARIAE, Yien M. KUO & Albert E.BIANCO. Imperial College, Prince Consort Road,London SW7 2BB,UK.

In a bovine model of onchocerciasis we have demonstrated that it is possible to induce high levels of protection against *Onchocerca lienalis* microfilariae by immunistion, whereas animals harbouring patent infections remain non-immune. Because serum from immunised cattle was able to confer protection by passive transfer, we set out to analyse antibody specificities in immune animals to identify putative targets of the host protective response. Immunoblot analysis revealed that sera from immune animals recognized a set of stage-specific polypeptides in the range of 10-15kD in microfilarial extracts. One of these molecules, that also arises as a major component of adult female worms, is conserved among *Onchocerca* spp., but is absent from *Brugia malayi*. A cDNA clone derived from an *Onchocerca volvulus* expression library was selected on the basis of its specific reactivity with antibodies from immune, but not infected animals. By immunoblot analysis of antigens from *O.lienalis* and *B.malayi* microfilariae, anti-E30 serum was shown to react with a 15kD antigen which was absent from adult worm extracts. By immunoelectron microscopy the 15kD molecule appears to be localized on the surface of *B.malayi* microfilariae but not on the surface of the adults. In adult female worms the antigen is on the surface of *in utero* microfilariae and in the uterus itself.

O 107 NON-ANTIBODY MEDIATED IMMUNITY AGAINST GAMETOCTYES IN A SIMIAN MALARIA SYSTEM P.CYNOMOLGI IN THE TOQUE MONKEY, Mendis K N, Maotunne T de S, Del Giudice G, & Carter R, Department of Parasitology, Faculty of Medicine, University of Colombo, Sri Lanka, As in P.vivax infections in man, P.cynomolgi infections induce anti-gamete immunity in its natural host the toque monkey, Macaca sinica. During the first 4 days of patent infections in spleen intact animals when anti-gamete antibodies were rising, but had not reached blocking levels the infectivity of gametocytes was enhanced by antibodies. Transmission blocking effects were detectable in the sera about a week after patency, and were completely lost after 2-3 months following self-cure. In splenectomised animals, in addition to these antibody mediated effects, an antibody independant mechanism of immunity against gametocytes was evident. In splenectomised animals at the peak of parasitaemia 5-7 days after patency, 'crisis' occured. At 'crisis' all intra-erythrocytic parasites including gametocytes appeared morphologically abnormal and the infectivity of the infection to mosquitoes dropped to zero. Gametocytes obtained from the 'crisis' period were non-infectious to mosquitoes even when washed and resuspended in normal (non-immune) monkey serum. Further, healthy gametocytes from a heterologous infection were rendered non-infectious when cultured in-vitro for 3 hours in medium containing 20% crisis serum, suggesting that soluble non-antibody mediators of immunity present in crisis serum 'inactivates' intraerythrocytic gametocytes. It is possible that these effects are mediated by cytokines since culture supernatants from monkey peripheral blood lymphocytes stimulated in-vitro with lipopolysaccharide also had a similar neutralising effect on intra-erythrocytic gametocytes in-vitro, rendering them non-infectious to mosquitoes.

O 108 TUFTSIN : EFFECTS ON THE PHAGOCYTIC AND MICROBICIDAL FUNCTION OF MACROPHAGES IN LEPROSY

RAO, D.N., DEPARTMENT OF BIOCHEMISTRY, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, NEW DELHI-110 029, INDIA.

Human peripheral blood monocytes/macrophages from normal donors, tuberculoid leprosy (BT/TT) and lepromatous leprosy (BL/LL) patients were assayed for stimulated phagocytic and microbicidal responses, to the potent macrophage stimulating tetra peptide,"tuftsin", after varying periods (6 hr - 14 days) of in vitro cultures. While normal and BT/TT macrophage cultures showed progressive stimulation[1] of phagocytosis of S.aureus, $H_{37}Ra$ and M.leprae as well as microbicidal[2] activity against S.aureus and M.leprae, with increasing age of in vitro culture, while the BL/LL cultures failed to respond to tuftsin. These findings led us to postulate that BL/LL macrophages may have altered tuftsin receptor expression or have altered signal transduction mechanism.

References: (1) Ravi R. Iyer and Rao, D.N., Revised Manuscript submitted to Infection and Immunity, 1988.

(2) Ravi R. Iyer and Rao, D.N., Revised Manuscript submitted to Infection and Immunity, 1988.

O 109 CONSERVATION OF A HUMAN GROWTH HORMONE-LIKE FACTOR IN THE TAPEWORM GENUS SPIROMETRA. C. K. Phares and J. Shaffer, Dept. of Biochem., Univ. of NE Med. Ctr., Omaha, NE 68105. K. Hirai, Dept. of Parasitol., Ehime Univ. School of Med., Ehime, Japan. Infections of mice with plerocercoids of either Spirometra mansonoides or S. erinacei result in enhanced growth of the host. The growth factor from S. erinacei does not stimulate growth of hypophysectomized rats, whereas pleroceroid growth factor from S. mansonoides (PGF-M) is a potent stimulant in this model. Mueller (J. Parasit. 58: 872-875, 1972) reported that infections of rats with plerocercoids of S. erinacei 5 wks prior to infection with S. mansonoides failed to immunize rats against the growth-promoting effects of PGF-M. Therefore, PGF-M and PGF-E may have different biological and immunological characteristics. We directly compared PGF-M and PGF-E. Isoenzyme patterns showed marked differences in all nine of the enzymes studied. Both growth factors displaced human growth hormone (hGH) from its receptors but PGF-E had only one-half the activity of PGF-M. Western blot analysis using three distinct anti-hGH monoclonal antibodies (MAb) showed that PGF-M and PGF-E share all three of the epitopes of hGH recognized by these MAb. Western blot analysis using a polyclonal antiserum to partially-purified PGF-M showed that the antiserum identified multiple bands in the crude PGF-M preparation but only one band in crude PGF-E crossreacted. The fact that all the anti-hGH MAb and the anti-PGF antiserum identified a 27.5K glycoprotein in both preparations suggests that both species share an analog of hGH. Therefore, while there can be little doubt that S. mansonoides and S. erinacei are distinct, the hGH-like factor is conserved in both species.

O 110 IDENTIFICATION OF PUTATIVELY PROTECTIVE ANTIGENS OF *BRUGIA MALAYI* BY THEIR ABILITY TO STIMULATE SPLEEN CELLS OF IMMUNE MICE TO PRODUCE IL-3 *IN VITRO*, Mario Philipp and Neil Storey, Molecular Parasitology Group, New England Biolabs Inc. Beverly MA 01915

We have shown that ^{3}H-thymidine incorporation by an IL-3 dependent cell line (32D) could be used to identify filarial antigens that stimulate splenic T-cells in immune mice. The procedure is simple and has the advantage that it will, in all likelihood, detect stimulation by antigen of T-cells of both the Th1 and Th2 helper subsets. We have identified a highly antigenic fraction (70-100 kD) which is not mitogenic, i.e. does not stimulate IL-3 secretion by spleen cells of naive mice. The antigens contained in this fraction were isolated from PBS extracts of *B. malayi* adult worms by one-dimensional SDS-PAGE and blotted onto nitrocellulose. Strips containing material of different molecular weights were used in the IL-3 bioassay

O 111 **NONIMMUNE KILLING OF TRYPANOSOMA BRUCEI BY A MINOR SUBSPECIES OF HUMAN HIGH-DENSITY LIPOPROTEIN**, E.M. Tytler, D.R. Moore, M.Pierce, J.D. Esko and S.L. Hajduk, Department of Biochemistry, University of Alabama at Birmingham, School of Medicine, Birmingham, AL 35294

Trypanosoma brucei brucei is an important human pathogen of domestic cattle in sub-Saharan Africa and is closely related to the human sleeping sickness parasites, *T. b. gambiense* and *T. b. rhodesiense*. However, *T.b. brucei* is non-infectious to humans. The restriction of the host range of *T.b. brucei* results from the sensitivity of the parasite to lysis by toxic human high-density lipoproteins (HDL). Trypanosome lytic activity is not a universal feature of all human HDL particles but is associated with a minor subclass of HDL. The trypanosome lytic factor (TLF) has been purified 8,000 fold and consists of a heterogeneous subclass of HDL which has a relative molecular weight of 490,000 a buoyant density of 1.21-1.24 g/ml and a particle diameter of 15-21 nm. TLF contains apolipoproteins AI, AII, CI, CII, CIII and at least three unique proteins of 92, 72 and 45 KDa. Incubation of purified ^{125}I labelled TLF with *T. brucei* at 4 °C suggests that TLF binding is saturable and is receptor mediated. Reconstituion studies are underway to identify the protein components of TLF involved in binding and trypanosome lysis.

O 112 A POSSIBLE ROLE OF INTERFERON AND OTHER CYTOKINES IN THE RESISTANCE OF CATTLE AGAINST THE RICKETTSIAE COWDRIA RUMINANTIUM DURING VACCINATION, John J. Wérenne[1], A.L.W. de Gee[2] and P. Totté[1] (1. Faculty of Sciences, Université Libre de Bruxelles, Belgium. 2.Department of Infectious Diseases, University of Florida, Gainesville, USA and Veterinary Research aboratory, Causeway, Zimbabwe; Present adress: Gezondheids dienst voor Dieren Noord Nederland, Drachten, The Netherlands/ in vivo study made in Zimbabwe under a contract between the "USAID" N° AFR 0000-C-6003-00 and the Government of Zimbabwe).
Heartwater, a deadly disease of cattle due to Cowdria ruminantium, is prevalent in the African continent, south of the Sahara, where the Amblyoma ticks are vectors. A primitive procedure of vaccination (intravenous administration of contaminated blood), confers a relative protection against the disease.We have studied a dozen of cows experimentally infected in such a way. Without antibiotic treatment only part of the animals survive. In the plasma of those animals contrary to others, an antiviral activity can be detected by the classical test of reduction of the cytopathic effect of VSV early after infection. Part of the antiviral activity could be ascribed to interferon, but other cytokines are also involved.

O 113 IMMUNOGENICITY OF A RECOMBINANT P. falciparum CS PROTEIN: LACK OF GENETIC RESTRICTION AND IDENTIFICATION OF NON-VARIANT T-CELL EPITOPE. Fidel Zavala, Philip Barr, Ruth Nussenzweig and Victor Nussenzweig, Department of Medical and Molecular Parasitology and Pathology, New York University Medical Center and Chiron Corporation, Emeryville. A recombinant CS protein encoding the sequence comprised between amino acid 40 and 340 of the native P. falciparum CS protein was used to immunize mice of different genetic background and H-2 haplotypes. All 11 strains tested produced a strong antibody response which recognized the repeated epitope $(NANP)_3$ and the parasites. More importantly, this recombinant protein was able to induce a secondary antibody response in sporozoite immunized mice.

We also identified the T cell epitopes present in the recombinant protein. It was found that at least two different epitopes were recognized by the immune T cells. One of them is a previously described polymorphic epitope (Th2r), the other is located between the repeat domain and Th2r. Though this newly described epitope displays one aminoacid substitution in different parasite isolates, this polymorphism does not impair the capacity of T cells to recognize the different variants.

O 200 EXPRESSION OF RIBOSOMAL RNA GENES IN **PLASMODIUM FALCIPARUM,** Talat Afroze, Dorothy Shippen-Lentz and Anne C. Vezza, Department of Medicine, University of Alabama at Birmingham, AL 35294. The ribosomal RNA genes of P. falciparum are unusual as compared to those of other eukaryotes. Previous work has shown that the parasite has only 4 to 8 copies which are dispersed throughout the genome. Based on restriction endonuclease digestion analyses, other labs have categorized these genes into 2 to 3 distinct classes which differ in their coding regions. Unlike previous reports, we have found that there are at least 5 to 6 unique rRNA gene classes in the parasite's genome which differ not only in their coding sequences but copy number as well. Sequence analyses of the 5.8S region of various classes suggests that most represent low copy pseudogenes. In addition, restriction endonuclease analysis indicates that some of these genes are linked. Only Class II genes are expressed during the erythrocytic stage of development. Time course experiments have shown that the transcription of Class II genes does not occur continuously throughout the asexual life cycle; instead, there is a lag time of 12 to 20 hours postinvasion.

We have also shown that the parasite contains only three 5S rRNA genes which have identical coding regions; however, their transcripts differ at the 3' end by 1 to 2 additional nucleotides. In order to determine if the heterogeneity was the result of aberrant transcription termination or gene specific termination, circular plasmids, containing different cloned plasmodial 5S genes, are being transcribed in vitro using an S-100 extract from human kidney cells. Preliminary studies indicate that similar size products are transcribed from each gene.

O 201 MOLECULAR CLONING OF THE 6-PHOSPHOGLUCONATE DEHYDROGENASE GENE FROM *TRYPANOSOMA BRUCEI* BY COMPLEMENTATION IN *E.COLI*.

Michael P. Barrett & Richard W.F. Le Page, Department of Pathology, University of Cambridge, Tennis Court Road. CAMBRIDGE CB2 1QP.

In order to study the structure and regulation of genes encoding house-keeping enzymes in *Trypanosoma brucei* we have developed a system of cloning by complementation in *E.coli* and used it to identify the structural gene for 6-phosphogluconate dehydrogenase (*gnd*). A *T.brucei* genomic library was constructed in a family of *E.coli* expression vectors and used to transform an *E.coli gnd* mutant . After prolonged incubation at 28°C on minimal selective medium several colonies grew. The colonies were found to harbour recombinant plasmids carrying trypanosome DNA of various lengths. Southern analysis against EcoR1 digested total trypanosome DNA indicated that the inserts hybridized to a small number of fragments. The plasmid carrying the smallest insert was chosen for further analysis; transformation of *E.coli gnd* point and deletion mutants restored growth on selective medium at 28°C and 6-phosphogluconate dehydrogenase activity was present in these transformants. Complementation of the deletion mutant provides strong evidence that we have identified the trypanosome *gnd* structural gene rather than a suppressor function.

O 202 GENOMIC ORGANISATION AND SEQUENCE ANALYSIS OF A REPEATED DNA SEQUENCE FROM *THEILERIA PARVA*, Howard A Baylis, Basil A Allsopp, Sarjit K Sohal and Mark Carrington, Department of Biochemistry, University of Cambridge, Tennis court Road, Cambridge CB2 1QW, UK.

Previous work resulted in the isolation of a 620 bp. fragment of repetitive DNA from *Theileria parva* and its application to stock discrimination (Allsopp BA and Allsopp MTEP 1988, Molecular and Biochemical Parasitology 28, 77-84). A plasmid clone of this fragment was used to isolate further clones from a λ library of *T. parva* Muguga genomic DNA. Analysis of five clones by restriction mapping and Southern analysis revealed that the 620 bp. sequence was part of a larger, approximately 2 Kbp., repeat present as tandem arrays in the genome. The DNA sequence of a region containing copies of the 2 Kbp. repeat and sequences flanking the tandem array has been determined. The repeat contains a large open reading frame which, with other features of the sequence, will be discussed. The function (if any) of this element is unknown; no rearrangements, as detected by RFLP patterns, have as yet been observed during *in vitro* culture of *T. parva* lymphocytes. Transcriptional studies are underway.

O 203 DNA-MEDIATED TRANSFORMATION OF LEPTOMONAS SEYMOURI.

Vivian Bellofatto, David Sherman, George A.M. Cross, The Rockefeller University, New York, NY 10021

Using a plasmid containing the bacterial chloramphenicol acetyl transferase gene we have assayed for transient expression of DNA introduced into Leptomonas seymouri by a variety of methods. Recent work will be presented.

O 204 RNA-EDITING IN TRYPANOSOME MITOCHONDRIA, Rob Benne, Hans van der Spek, Janny van den Burg and Paul Sloof, Laboratory of Biochemistry, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

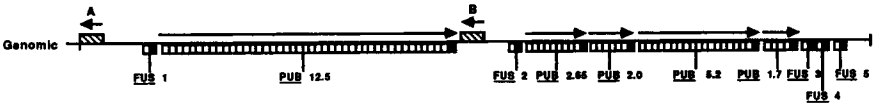
The nucleotide sequence of trypanosome mitochondrial transcripts differs at certain sites from that of the corresponding genomic sequence as a result of a novel U-insertion/deletion process (RNA-editing). In general, RNA-editing repairs translational defects present in the genomic sequence, such as frameshifts or absence of an AUG-translation initiation codon. We have determined the nucleotide sequence of cDNAs derived from MURF3, MURF2, coxIII, coxII and NDI transcripts of Crithidia fasciculata. RNA-editing creates an in-frame AUG-initiation codon in coxIII and MURF2 RNAs and repairs a frameshift in MURF3 and coxII RNAs. In MURF3 RNAs a genomically encoded, putative initiation codon is disconnected from the reading frame by RNA-editing. Most cDNAs have an edited poly[A] tail, which varies in sequence even for cDNAs derived from the same gene. Outside the areas mentioned, the RNA sequences match those of the genome. Surprisingly, no evidence of editing was found in NDI cDNAs, which lack a genomic initiation codon. The MURF3, MURF2 and coxIII cDNA collection also contains unedited and partially edited cDNAs in which a 3' edited segment is combined to an unedited 5' region. Other cDNAs are truncated and have their 5' end at an editing site. The latter two categories of cDNA may be derived from RNAs that are intermediates of an editing process that moves from 3' to 5' during which breaks are introduced at the sites to be edited, followed by U-insertion/deletion and religation. No evidence for a template encoding edited MURF3 sequences could be found in total C. fasciculata DNA or RNA.

O 205 EXTENSIVE RNA EDITING OF A G-RICH MITOCHONDRIAL TRANSCRIPT IN *TRYPANOSOMA BRUCEI*, G. Jayarama Bhat and Kenneth Stuart, Seattle Biomedical Research Institute, Seattle, WA. 98109

A post-transcriptional RNA editing process occurs in *Trypanosoma brucei* which can substantially alter the genomically encoded message. During this process uridines are either inserted or deleted at multiple positions along the length of the transcript. Previous studies have shown that a G+C rich area of mitochondrial DNA 5' to the cytochrome *b* gene encodes cytochrome oxidase subunit III transcripts which are extensively (>50%) edited. We have now examined transcripts from a similar G+C rich region 3' to the cytochrome *b* gene. This region spans approximately 400 nucleotides and transcripts from this region are also extensively edited. Four discrete size classes of polymerase chain reaction (PCR) products have been detected. One of these occurs in a stage specific fashion. These products have been cloned and sequenced.

O 206 IN VITRO 3' END PROCESSING OF RNA IN TRYPANOSOME NUCLEAR EXTRACTS, Gregory A. Buck, Suzanna L. Short and Tadeusz A. Zwierzynski. Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 23298. We have established nuclear extracts from T. cruzi that are competent for RNA polyadenylation and several other 3' RNA modification reactions. Our extracts efficiently tail synthetic RNAs by the addition of 10-20 AMP residues. This activity is sensitive to protease treatment, Micrococcal Nuclease treatment and to short incubations at elevated temperatures suggesting that the activity is mediated by a temperature sensitive protein-RNA complex. The polyadenylation activity is limited to the nucleus; cytoplasmic S100 fractions inhibit the reactions and may contain specific inhibitory factors. We believe that this activity may be responsible for in vivo mRNA polyadenylation because of its nuclear location and because many of its properties resemble the polyadenylation systems of higher eukaryotes. We have also identified and partially characterized several other RNA 3' end modification activities in these extracts, i.e. a terminal uridyl transferase, a terminal cytidyl transferase and a 3' specific exonuclease. Finally, we identified a very active cytidine deaminase activity that converts CTP to UTP in solution.

O 207 DIVERGENT TRANSCRIPTION FROM THE UBIQUITIN GENE CLUSTER OF TRYPANOSOMA CRUZI, Chung,S.H.[1], Swindle,J.[1,2], [1]Department of Microbiology and Immunology, University of Tennessee, Memphis, TN. [2]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.. The polyubiquitin (PUB) genes of Trypanosome cruzi strain CL are organized as a single gene cluster. As illustrated below the PUB genes are all transcribed in the same 5' to 3' orientation (the solid arrows indicate the direction of transcription). Recently we have identified two additional genes (identified as A and B in the diagram below) which are transcribed from the ubiquitin gene cluster but in the opposite 5' to 3' direction. The transcription map for the A and PUB12.5 genes indicates that the two genes are divergently transcribed from promoters within the 2.0Kbp separating the two genes. Similarly transcription of the B and PUB2.65 genes appears to be divergent initiating from promoters within the 1.5Kbp to 2.0Kbp separating the two genes.



O 208 REGULATED EXPRESSION OF THE FRUCTOSE BISPHOSPHATE ALDOLASE GENES OF TRYPANOSOMA BRUCEI

Christine E. Clayton and *S. Vijayasarathy
The Rockefeller University, 1230 York Ave, New York, NY 10021 and *Stanford University Medical Center, Stanford, CA 94305, USA

While in the mammalian host, trypanosomes derive all their energy from substrate-level phosphorylation during glycolysis. Adaptation to the tsetse fly environment involves extensive mitochondrial elaboration including the building of a network of cristae and the appearance of citric acid cycle enzymes and cytochromes; at the same time, the rate of glycolysis is thirty-fold reduced.

Fructose bisphosphate aldolase is thirty times more abundant in bloodstream trypanosomes than in the "procyclic" insect forms. The corresponding mRNA levels reflect this regulation. The regions around the aldolase genes have been sequenced. Each aldolase locus contains two aldolase genes arranged as tandem repeats, and produces at least three other polyadenylated transcripts, in the same transcriptional orientation as aldolase but, unlike aldolase, expressed at a low level throughout the life cycle. The additional transcripts have open reading frames that could encode small proteins. The sequenced regions are all being thoroughly analysed by measurement of transcription in isolated trypanosome nuclei. The data so far are consistent with transcription of the aldolase and associated genes as a poly-cistronic precursor. This means that some regulation must be effected post-transcriptionally at the level of mRNA processing.

O 209 ISOLATION AND CHARACTERIZATION OF THE RNA POLYMERASE LARGEST SUBUNIT GENES FROM TRYPANOSOMA BRUCEI: THE IDENTIFICATION OF AN ADDITIONAL POL II LOCUS. R. Evers, A. Hammer, W. Jess, J. Köck and A.W.C.A. Cornelissen, Max-Planck-Institut für Biologie, 7400 Tübingen, F.R.G.

The variant surface glycoprotein (VSG) genes in *T.brucei* are transcribed in an alpha-amanitin insensitive way. This led to the speculation that VSG is transcribed by RNA pol I or by an additional alpha-amanitin resistant RNA pol II (1,2). In order to decide between these two possibilities, we have cloned and sequenced the genes encoding the RNA polymerase largest subunit genes in *T.brucei* (3,4).

Besides the genes coding for RNA pol I and III, two loci were identified encoding a RNA pol II sequence. Both genes were almost identical at the amino acid level. Interestingly this situation was only found in species performing antigenic variation. Only a single copy could be detected in all species analysed and not exhibiting antigenic variation. These data indicate that an additional RNA pol II might play a role in the transcription of VSG genes.

Another remarkable feature of the RNA pol II genes is the presence of a modified C-terminal extension. In all eukaryotes analysed thusfar, the C-terminal domain of RNA pol II consists of a tandemly repeated heptapeptide sequence. This repeat structure is absent in the trypanosomal domain, which moreover is rich in acidic aminoacids. This situation is not unique for *T.brucei*, since it is also found in the distantly related species *C.fasciculata*. Data supporting the results described above will be presented. References: 1. Laird et al. (1985): *Nucl Acids Res* 13, 4253; 2. Kooter and Borst (1984) *Nucl Acids Res* 12, 9457; 3. Köck et al. (1988): *Nucl Acids Res* 16, 8753; 4. Evers et al. (1988) *Cell*, in press.

O 210 RNA EDITING IN KINETOPLASTID MITOCHONDRIA, Jean E. Feagin and Kenneth Stuart, Seattle Biomedical Research Institute, Seattle, WA 98109-1651

RNA editing is a recently discovered process which alters transcripts, probably posttranscriptionally, by the addition of uridines which are not encoded in the gene and, less frequently, by the deletion of encoded uridines. Transcripts from many of the mitochondrially-encoded genes of several kinetoplastid protozoans are edited and some editing in *Trypanosoma brucei* is developmentally regulated. Editing corrects genomic frameshifts, extending open reading frames, and creates initiation and termination codons, thus having profound effects on the predicted protein sequences. The cytochrome oxidase III transcript of *T. brucei* is so extensively edited, with over 55% of its sequence due to editing, that the gene itself is unrecognizable. Analysis of edited RNA and cDNA sequences suggests that transcripts are edited in the 3' to 5' direction and that multiple editing events may be necessary at some sites to reach the final sequence. It further suggests that steps involved in editing include cleavage of substrate RNA, addition of uridines, (possibly) trimming of excess uridines, religation, and translocation of the (hypothetical) editing complex to the next site to be edited. Studies of the *T. brucei* cytochrome oxidase III transcripts are now focused on determining the 5' end sequence, analyzing putative editing intermediates, and comparison of editing among stocks and species.

O 211 **THE CRITHIDIA FASCICULATA MINI-EXON GENE LOCUS: TARGET FOR A RAPIDLY REARRANGING RETROTRANSPOSON,** **[1]Abram Gabriel, [1]Tim J. Yen, [2]David C. Schwartz, and [1]Don W. Cleveland, [1]Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore MD 21205; [2]Department of Embryology, Carnegie Institution of Washington, Baltimore MD 21210**

Multiple copies of the tandemly arrayed Crithidia fasciculata mini-exon genes are interrupted at a specific site (the 5' splice junction) by an ~3.6 kb genetic element with properties of a non-long terminal repeat retrotransposon. Structural and sequence features of the Crithidia Retrotransposable Element (CRE1) include: a) an 1141 amino acid open reading frame with greatest similarity to retroviral reverse transcriptases; b) 29 bp terminal target site duplications; c) variable length 3' poly A stretches; d) a precise insertion site within the mini-exon gene repeat. CRE1 is present at a copy number of ~10 per genome while the mini-exon genes are present at ~500 per genome.

Cloned lines of C. fasciculata from the same population show unique organizations of CRE1 within the mini-exon locus, as well as the presence of CRE1 and mini-exon genes on variable size chromosomes. Subclones of cloned lines demonstrate a rearrangement frequency for CRE of ~1% per generation. Current work centers on determining the mechanisms of CRE1 rearrangements.

O 212 POST TRANSCRIPTIONAL CONTROL OF GENE EXPRESSION IN TRYPANOSOMA BRUCEI, Graham, S.V., McCulloch, R. and Barry, J.D. Institute of Genetics and Wellcome Unit of Molecular Parasitology, University of Glasgow, Church St., Glasgow G11 5JS, U.K. Little is known about the process of gene expression and its control in the parasitic protozoan Trypanosoma brucei. However, several unusual features of transcription in this organism have been identified. Firstly, many genes tandemly arrayed in the genome are co-transcribed to yield a polycistronic primary transcript. Thus, several genes may occupy one transcription unit and be under control by the same promoter, making transcriptional control of individual genes difficult. Secondly, polycistronic primary transcripts appear to be processed to yield individual, mature mRNAs by trans-splicing of a discontinuously transcribed spliced-leader sequence and by polyadenylation. These peculiarities in gene expression argue for a major role for post transcriptional control of gene expression in trypanosomes. Specifically it has been suggested that regulation of rates of decay of mature transcripts may constitute an important control mechanism. We have investigated expression of several trypanosome mRNAs, including both "housekeeping" and more specialised transcripts using run-on analysis of nascent transcripts, steady-state Northern blot analysis and direct analysis of rates of decay of individual mRNAs. We will present data comparing transcription rates and rates of decay between "housekeeping" mRNAs and life cycle/stage-specific transcripts. The role of post transcriptional control of gene expression in maintaining appropriate levels of gene products during the parasite life cycle will be discussed.

O 213 AT LEAST THREE CLASSES OF RNA POLYMERASES DIRECT TRANSCRIPTION IN TRYPANOSOMES. E.J.M. Grondal, K. Kosubeck and A.W.C.A. Cornelissen, Max-Planck-Institut für Biologie, 7400 Tübingen, FRG.
African trypanosomes show a number of remarkable features in their transcription process. Among them the transcription of VSG genes is still puzzling. Nuclear run on experiment have shown that transcription of VSG genes is completely resistant to alpha-amanitin, a potent inhibitor of RNA pol II, whereas transcription of other protein coding genes is inhibited by the drug (1).

Speculations about the contribution of RNA pol I or a modified RNA pol II arised (2,3) but are thusfar not supported by protein data. We therefore started to purify RNA polymerases from *T.brucei*. RNA polymerase activities could be separated into three forms (I-III) with typical eukaryotic characteristics. Differences were observed in the amanitin sensitivity of RNA pol II which is much lower than that of other eukaryotic RNA pol II's and is close to that of RNA pol III. Both forms can, however, be discriminated by other properties, eg. template specificity and ion requirement. Antibodies against a fusion protein containing the C-terminal extension of the largest subunit of *T.brucei* RNA pol II, reacted with protein fractions of the RNA pol II peak, supporting the initial classification.

We were able to allocate two activities to the RNA pol II class: one for VSG genes and one for other protein-coding genes. The evidence will be summarized. References: 1. Laird et al. (1985) *Nucl Acids Res* 13, 4253; 2. Kooter and Borst (1984) *Nucl Acids Res* 12, 9457; 3. Shea et al. (1987) *Cell* 50, 603.

O 214 **MITOCHONDRIAL RIBOSOMAL RNA ACCUMULATION IS DEVELOPMENTALLY REGULATED IN TRYPANOSOMA BRUCEI AT THE LEVEL OF RNA STABILITY,** S.L. Hajduk, E.M. Michelotti, M.E. Harris and K.I. Bertrand, Department of Biochemistry, University of Alabama at Birmingham, School of Medicine, Birmingham, AL 35294
Mitochondrial activities are developmentally regulated during the life-cycle of *Trypanosoma brucei*. Both the long slender and short stumpy developmental stages in the mammalian bloodstream repress mitochondrial activities and derive ATP entirely by glycolysis. Procyclic, insect developmental stages in the insect vector or in culture at 26°C have fully functional mitochondria. During the differentiation from the bloodstream long slender stage to the insect procyclic stage the steady state levels of 9S and 12S rRNA increase 60 fold. Ribosomal RNA transcription initiates at least 1.2kb upstream of the 5' end of the 12S rRNA in both long slender and procyclic trypanosomes. Measurements of the rate of rRNA transcription initiation in pulse labelled cells indicates that the mitochondrial transcription rate remains constant during the trypanosome developmental cycle. This suggests that mitochondrial rRNA levels are controlled during the developmental cycle by a mechanism which modulates the stability of the mature rRNAs. A comparison of the sequence of the 9S rRNA gene and the RNA revealed that the 9S rRNA sequence differs from the 9S gene by the addition of 6 uridine residues near the 3' end of the transcript. The presence of added uridines allows the formation of a stable stem-loop structure at the 3' end of the 9S rRNA. We propose that the formation of this structure is developmentally regulated and contributes to the stability of the mitochondrial rRNAs.

O 215 **TRANSCRIPTION AND RNA EDITING IN ISOLATED MITOCHONDRIA FROM TRYPANOSOMA BRUCEI,** M.E. Harris and S.L. Hajduk, Department of Biochemistry, University of Alabama at Birmingham, School of Medicine, Birmingham, AL 35294
Some trypanosome mitochondrial transcripts differ from the genes encoding them by the addition or deletion of uridine residues. This process, termed RNA editing, can be both extensive and functionally significant. RNA editing has been implicated in the correction of frame-shifts in protein coding sequences, in the formation of translation initiation codons and in the developmental control of mitochondrial RNA stability. In order to study the biochemical mechanisms underlying RNA editing we have developed a system using isolated mitochondria which allows accurate transcription initiation and processing. Hybridization of mitochondrial RNA, pulse labelled *in vitro* with ^{32}P-GTP, to Southern blots of kinetoplast DNA suggests that transcription and processing of the mitochondrial rRNA transcripts is similar to that seen in intact trypanosomes. Incubation of mitochondria in the absence of CTP inhibits RNA synthesis by >90% as measured by ^{32}P-GTP incorporation. RNA editing appears uneffected by CTP deprivation and ^{3}H-UTP incorporation into mitochondrial RNA proceeds at a rate of 32 pmoles/min/mg mitochondrial protein. The incorporation of ^{3}H-UTP was linear for 60 minutes in the absence of mitochondrial transcription and was ATP independent. These results demonstrated that RNA editing was a post-transcriptional event.

O 216 DEVELOPMENTAL REGULATION OF RNA EDITING IN THE *TRYPANOSOMA BRUCEI* MURF3 GENE, Donna J. Koslowsky and Kenneth D. Stuart, Seattle Biomedical Research Institute, Seattle, WA. 98109

In trypanosome mitochondria an RNA editing process post-transcriptionally alters the nucleotide sequence of transcripts by insertion and/or deletion of uridine residues at specific sites. This editing process can extend open reading frames, correct frame shifts and create in frame initiation and termination codons. In *T. brucei*, RNA editing is developmentally regulated for some genes and for these RNAs is restricted to the procyclic life-cycle stage (insect form) when a fully functional mitochondrion is present. However, this pattern is not seen in the *T. brucei* MURF3 gene. Analysis of cDNAs derived from both bloodstream and procyclic transcripts indicates that the editing process is extensive in both life stages with editing occurring at both the 5' and 3' ends. Northern blot analysis of total RNA using edited oligo probes indicate that MURF3 mRNA transcripts are approximately 200 nucleotides larger in bloodstream form RNA than in procyclic form RNA. RNA sequence analysis indicates that an in frame AUG codon is created by uridine addition in Bloodstream RNA. This contrasts with the RNA editing patterns seen by van der Spek et al. in *Crithidia fasciculata* and by Shaw et al. in *Leishmanis tarentolae*. In *C. fasciculata* an in frame AUG is moved out of frame by the addition of 22 uridines at the 5' terminus. In *L. tarentolae* no in frame AUG was detected after RNA analysis of the 5' terminus. The implications of these results will be presented.

O 217 PURINE METABLISM OF TOXOPLASMA GONDII, Edward C. Krug, Randolph L. Berens, J. Joseph Marr. Depts. of Med. and Biochem., Univ. of CO. Health Sciences Center, Denver, CO 80262. We have studied the incorporation and interconversion of purines into the nucleotide pools and into RNA and DNA. The purine bases hypoxanthine, xanthine, guanine, and adenine are incorporated at 9.2, 6.2, 5.1, and 4.3 pmoles/10^7 cells/hour respectively. The purine nucleosides adenosine, inosine, guanosine, and xanthosine were incorporated at 110, 9.0, 2.7, and 0.3 pmoles/10^7 cells/hour respectively. Guanine, xanthine, guanosine, and xanthosine labeled only guanine nucleotides. Inosine, hypoxanthine, and adenine labeled both nucleotide pools at nearly equal ratios. Adenosine labeled the adenine nucleotide pools at a 10 fold higher rate than the guanine nucleotide pools. Incorporation of the purines into both RNA and DNA roughly paralleled the incorporation into the nucleotides. Analysis of cytosolic enzymes revealed a nucleoside kinase activity for only adenosine, and no nucleoside phosphotransferase or hydrolase activities. Phosphorylase activities were detected for inosine and guanosine. Cytosolic deaminase activities were found for adenine and guanine but not for adenosine. Phosphoribosyltransferase activities were detected for all four purine bases. Collectivelly these data indicate that adenosine is a sufficient purine source and the prefered one.

O 218 INTRODUCTION AND EXPRESSION OF TRANSFECTION VECTOR pALTI-I IN LEISHMANIA ENRIETTII . Avraham Laban and Dyann F. Wirth, Department of Tropical Public Health, Harvard School of Public Health , Boston MA 02115

Transfection of nucleic acids into cells is one of the most powerful tools in the field of molecular biology. We developed a system that allows us to introduce and to express genes in protozoan parasite *Leishmania enriettii*. In this system the immediate upstream region of the α-tubulin gene was inserted in front of reporter gene. The thought was to provide the reporter gene with regulatory elements for transcription and for translation from *Leishmania enriettii* . To monitor the level of expression in this transfection system we used CAT gene as the reporter and subsequently assayed CAT enzyme activity. This is a reliable, accurate and sensitive assay. The fusion of the CAT gene and the upstream region of α-tubulin was cloned into the *E.coli-K12* vector pBLUESCRIPT. The delivery of the construct, now called pALTI-I, into parasites was mediated by electroporation. Optimal transfection was achieved in high ionic electroporation buffer with 25μF capacitor and voltage of 3000V/cm. Under these conditions ~10% of the chloramphenicol was acetylated by CAT activity in lysates of 10^8 parasites. The same level of activity was detected for at least five days. The plasmid pALTI-I carries 800 bp of the upstream region of α-tubulin. To define which part of this region is necessary for CAT gene expression, nested deletions were made. Reduction of the region to 130 bp did not decrease the CAT activity in parasite lysates. Complete removal of the region reduced CAT activity to very low levels.

The transfection system described above is of major importance. Using this system we will define important DNA sequences for transcription, RNA processing and translation.

O 219 A TRANSCRIPTIONAL ANALYSIS OF THE TRYPANOSOMA BRUCEI HSP83 GENE TANDEM ARRAY, J.C. Mottram[1,2] and N. Agabian[2], [1]Wellcome Unit of Molecular Parasitology, Institute of Genetics, Glasgow University, UK and [2]Dept of Pharmaceutical Chemistry, UCSF, San Francisco, CA 94143. Many trypanosome genes, including calmodulin and tubulin, are arranged in tandem arrays and are transcribed into polycistronic pre-mRNAs which are subsequently trans-spliced and polyadenylated to give the mature mRNA. The elements controlling the transcription of these genes, however, are not well understood and indeed no polymerase II promoters have yet been identified. In higher eukaryotes the heat shock genes are among the best studied, and we have therefore cloned the trypanosome Hsp83 gene and initiated an analysis of its transcription. 10-12 copies of the gene are found in tandem, the 2.8kb repeat units of which are transcribed to give 2.2kb mRNAs which have the spliced leader at their 5' end. We have cloned and sequenced one of the repeat units and find that there is 218nt of intergenic sequence between the polyA addition site and the 3' splice site of the adjacent downstream Hsp83 gene. We have also cloned the region immediately 5' to the first repeat unit of the array and have investigated the site of transcription initiation by nuclear run on experiments. Unlike the situation in other trypanosome genes which are organised in tandem arrays it appears that the unique 5' flanking region of the Hsp83 gene cluster is not transcribed and that transcription initiation may occur from within the intergenic regions.

O 220 SYNTHESIS OF TRYPANOSOME hsp70 mRNA IS RESISTANT TO DISRUPTION OF TRANS-SPLICING BY HEAT SHOCK, Michael L. Muhich* and John C. Boothroyd, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305.

Synthesis of mRNA in trypanosomes involves an apparent trans-splicing reaction whereby a common 39 nucleotide mini-exon sequence is joined to the protein-coding exon of a mRNA precursor. We have previously shown (Muhich, M.L. and Boothroyd, J.C. (1988) Mol. Cell Biol., 8, 3837-3846) that the trans-splicing pathway of Trypanosoma brucei is sensitive to disruption by severe heat shock. Here we demonstrate that most, if not all, of the synthesis of heat shock protein 70 (hsp70) mRNA in T. brucei is resistant to the heat-induced disruption of splicing. The 5' ends of hsp70 mRNAs are shown to be identical for molecules synthesized at either normal or heat shock temperatures and in both cases, the 5' terminal mini-exon sequence is present. These results suggest that T. brucei has evolved a mechanism which directly compensates for the deleterious effects of heat shock on trans-splicing, one which allows for the continued, mini-exon dependent, trans-splicing of selected pre-mRNAs.

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O 222 ORGANIZATION AND TRANSCRIPTION OF THE BIFUNCTIONAL DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHETASE GENE FROM CRITHIDIA FASCICULATA, Dallas E. Hughes, Opeolu A. Shonekan and Larry Simpson, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

One of the two dihydrofolate reductase-thymidylate synthetase (DHFR-TS) genes from Crithidia fasciculata was isolated and characterized. There is a single low abundance polyadenylated transcript 3100 nt in size. One major miniexon splice site was identified by primer extension analysis. The 5' flanking region is divergently transcribed and has strong sequence similarities to a GC-rich consensus DHFR promotor as well as to other eukaryotic "housekeeping" gene promotor regions. A sequence downstream of the DHFR promotor consensus region is complementary to the 3' end of the medRNA from the same species, thereby suggesting a model by which the two separately transcribed RNAs may be juxtaposed for trans-splicing. Immediately 3' of the DHFR-TS gene there is a sequence that is common to all chromosomes of this and at least one other species of kinetoplastid (Leishmania tarentolae).

O 223 THE MITOCHONDRION OF LEISHMANIA CONTAINS FUNCTIONAL tRNAs WHICH APPEAR TO BE ENCODED BY NUCLEAR GENES, Yoshitaka Suyama, Agda M. Simpson, Homero Dewes, David Campbell and Larry Simpson, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024, and Department of Biology, University of Pennsylvania, Philadelphia, PA 19104

Cytoplasmic and mitochondrial-kinetoplast tRNAs isolated from Leishmania tarentolae were shown by 2D acrylamide gel electrophoresis to consist of 35-40 spots, most of which comigrated. Mitochondrial tRNAs (ktRNA) are functional as they could be individually acylated with specific amino acids (^{35}S methionine, ^{3}H leucine, ^{3}H glycine, ^{3}H tryptophan) by cytoplasmic and/or mitochondrial synthetase extracts, and the charged tRNAs identified by 2D gel electrophoresis. Both the mitochondrial and cytoplasmic fractions show two $tRNA^{met}$ species, one of which comigrates and one of which is unique to each fraction. This indicates that the mitochondrial tRNA fraction is free of cytoplasmic tRNA contamination. Mitochondrial tRNAs for glycine and leucine are identical with cytoplasmic tRNAs as evidenced by positive hybridization with a sequenced nuclear DNA clone from T. brucei. Hybridization of acrylamide purified ktRNA was negative with maxicircle or minicircle DNA and positive with nuclear DNA. However a ktRNA fraction isolated by DEAE chromatography contained additional RNA species which hybridized with maxicircle and minicircle DNA. These transcripts migrate ahead of tRNAs in acrylamide and are of unknown function. We conclude the mitochondrion of L. tarentolae contains a set of organelle-specific functional tRNAs as well as a subset of cytoplasmic tRNAs, which are encoded in the nucleus and transported into the mitochondrion, unlike the situation in human and fungal mitochondria in which the organelle genome encodes the complete set of tRNAs.

O 224 SEVERAL STRAINS OF LEISHMANIA TARENTOLAE CONTAIN SMALL "CHROMOSOMES" AS SEEN IN ORTHOGONAL FIELD GELS WHICH VARY IN COPY NUMBER AND CONSIST OF LINEAR DNA MOLECULES, Leonor Rovai and Larry Simpson, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

We have analyzed the chromosomal patterns in OFAGE, CHEF and FIGE of eight strains of Leishmania tarentolae (1) and have found that several strains contain small unique chromosome bands of multiple copy numbers, which we have termed "SNAs" for "small nucleic acids". Strain LEM 87 contains four SNA bands of different intensities. Strain LEM 115 wt cells do not contain any SNA bands, but after subjecting the culture to a nutrient starvation selection procedure, the karyotype of the surviving cells (LEM 115A) reproduceably changed in a defined manner: chromosome four vanished and one or two SNA bands appeared (SNA1 and SNA2), approximately 330 kb and 66 kb in size, respectively. SNA2 was not derived from chromosome four. The copy number of SNA2 varied greatly depending on culture conditions; however, since LEM 115 is not a clonal line, this variation may represent selection of preexisting cells rather than induction of copy number change. SNA 2 DNA hybridized with one of the low copy number small SNA bands from LEM 87. CD1 DNA, derived from a circular DNA from from L. donovani (Obtained from Dr. K. Stuart, Seattle Biomedical Research Institute), did not hybridize with any SNA from LEM 115A or LEM 87. SNA 2 from 115A contains linear DNA and telomere sequences. We are analyzing these unusual chromosome-like DNA species.

1. Gomez-Eichelmann, C., Holz, G., Beach, D., Simpson, A. and Simpson, L. (1988). Mol. Biochem. Parasitol. 27, 143-158.

O 225 TOPOISOMERASE II FROM T. BRUCEI, Phyllis R. Strauss*# and James C. Wang*, *Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138 and #Department of Biology, Northeastern University, Boston, MA 02115

Topoisomerases are involved in replication, transcription and recombination through their ability to alter the topology of DNA. Eukaryotic DNA topoisomerase II is an essential enzyme and catalyzes double-stranded DNA breakage, strand passage and rejoining. The gene TOP2 encoding eukaryotic DNA topoisomerase II from budding yeast, fission yeast, fruit flies and man has been cloned and sequenced . We report here the cloning and sequencing of the TOP2 gene from Trypanosoma brucei. The gene was identified from a lambda EMBL3 library by hybridization with the same oligonucleotide used to identify the gene for human topoisomerase II, which codes for the amino acid sequence MIMTDQD. It was subcloned into a phage-plasmid vector and sequenced by means of dideoxy chain termination. The gene is preceded by two potential trypanosome splice sites,$(C/U)_nNNAG$, at positions -19 and -65 and terminated by a polyadenylation signal. The start codon ATG follows the sequence TATACACA, which may provide a ribosome binding site. The deduced amino acid sequence indicates a 150 kD protein with homology in the first 75% of the protein for other type II topoisomerases. The oligonucleotide originally used to identify the gene is homologous to base pairs 1537-1550, which in the trypanosome gene codes for the amino acid sequence LIMTDQD. The amino acid sequence corresponding to the yeast active site RYI is located at base pairs 2308-2315. Blot-hybridization of restriction digests of genomic DNA indicates that there is one copy of TOP2 per genome. We conclude that T. brucei contains one single-copy gene for DNA topoisomerase II homologous to other type II topoisomerases. Supported by NIH GM24544.

O 226 CHARACTERIZATION OF A MULTICOPY DNA IN LEISHMANIA, Cynthia A. Tripp and Ken Stuart, Seattle Biomedical Research Institute, 4 Nickerson Street, Seattle, WA 98109

A 29 kb double stranded, circular DNA (CD1) has been isolated from L. donovani. Circular CD1-like molecules were also detected in other stocks within the L. donovani complex. In addition, sequences homologous to CD1 were found integrated in one of several chromosomes of other strains including a multicopy linear DNA (LD1) of L. b. braziliensis. CD1 has no homology to DHFR\TS and H region amplified DNA sequences of methotrexate resistant Leishmania. The restriction map of CD1 has been determined and CD1 has been cloned as a series of restriction fragments. The clones are being used to assess the diversity of sequences essential for the function and maintenance of CD1. Selected nucleotide analysis is in progress as are studies of CD1 transcription. These studies are designed to assess the significance of CD1 and its potential as a molecular cloning vehicle.

O 227 ORGANIZATION AND EXPRESSION OF NOVEL ORGANELLE DNA OF MALARIAL PARASITES. Akhil B. Vaidya, Kathleen Suplick, and Rama Akella, Hahnemann University, Philadelphia, PA 19102.

All malarial parasite species tested contain clusters of tandemly arrayed DNA sequences of 6 kb unit length (1). We have molecularly cloned the 6 kb units from two species, *Plasmodium yoelii* and *P. falciparum* (1,2). The *P. yoelii* sequence and its expression in erythrocytic stages have been examined in some detail, and results can be summarized as follows: 1. The 6 kb sequence is transribed in a complex manner with only a 0.5 kb region not represented in stable non-polyadenylated RNA. Two large transcripts, 1.6 and 1.1 kb long, originate from a slightly overlapping region, and a relatively minor RNA of 2.3 kb is recognized by most of the subfragments of the 6 kb sequence. A large number of small RNA molecules with discrete sizes ranging from 400 to 80 nucleotides are encoded by different regions of the 6 kb sequence. 2. The DNA sequencing of the entire 6 kb (3) revealed sequence similarity with two mitochondrial proteins, cytochrome c oxidase subunit I and cytochrome b, potentially encoded by the 1.6 and 1.1 kb RNAs, respectively. Some unusual processing or editing will be required to overcome numerous stop codons and reading frameshifts to generate mRNAs encoding these proteins. 3. No standard ribosomal RNA genes could be detected in the 6 kb sequence. However, two regions present on separate strands have remarkable similarity to the highly conserved peptidyl transferase domain of the large rRNA. These molecules will have to associate in *trans* to form a portion of the peptidyl transferase domain. This sequence had no homology with the published partial sequence of a 30 kb organelle-like circular DNA of *P. falciparum* (4). Our results strongly suggest that malarial parasites have a bimolecular organelle genome, one of which represented by a novel and non-conventional 6 kb tandemly arrayed DNA molecule. References: 1. Vaidya and Arasu, Mol. Biochem. Parasitol. 22, 249, (1987). 2. Suplick et al. MBP 30, 289, (1988). 3. Vaidya et al. MBP, in press, (1989). 4. Gardner et al. MBP 32, 11 (1988).

O 228 CHARACTERIZATION OF AN RNA VIRUS IN LEISHMANIA, Reitha S. Weeks[*], Giovanni Widmer[+], Jean L. Patterson[+],and Kenneth Stuart[*], [*]Seattle Biomedical Research Institute, 4 Nickerson St, Seattle, WA 98109; [+]Department of Microbiology and Molecular Genetics, Harvard Medical School, Division of Infectious Diseases, Children's Hospital, 300 Longwood Ave., Boston, MA 02115

RNA viruses were detected in promastigotes of two isolates of *Leishmania braziliensis guyanensis* as ethidium bromide staining 6kb bands in agarose gels or as an RNA-dependent RNA polymerase activity synthesizing large transcripts. The putative 6 kb viral genome, also present in four other *L. braziliensis* isolates, is single stranded RNA based on its sensitivity to RNase and alkali but not DNase. This RNA is linear, terminating in 5' phosphate and 3' hydroxyl groups based on end labelling experiments. Particles that are 32nm in diameter were isolated from cytoplasm in the 130S fraction of sucrose density gradients which contains the 6kb RNA. The physical characteristics and RNA content of the particles were examined under various conditions. The stability of the particle under various salt concentrations, ionic conditions, and pH were examined. Portions of two 6kb viral RNAs have been cloned as cDNAs and sequenced. No homology to other viral RNAs has been detected. The 6kb RNA from two isolates are not identical. RNA from various isolates is being examined with cloned probes to determine the extent of homology among the 6kb nucleic acids. Also see Tarr, P.I., R.F. Aline Jr., B.L. Smiley, J. Scholler, J. Keithly & K. Stuart. 1988. PNAS (in press).

O 229 CHARACTERIZATION OF AN RNA VIRUS IN LEISHMANIA, Reitha S. Weeks*, Giovanni Widmer+, Jean L. Patterson+,and Kenneth Stuart*, *Seattle Biomedical Research Institute, 4 Nickerson St, Seattle, WA 98109; +Department of Microbiology and Molecular Genetics, Harvard Medical School, Division of Infectious Diseases, Children's Hospital, 300 Longwood Ave., Boston, MA 02115
RNA viruses were detected in promastigotes of two isolates of *Leishmania braziliensis guyanensis* as ethidium bromide staining 6kb bands in agarose gels or as an RNA-dependent RNA polymerase activity synthesizing large transcripts. The putative 6 kb viral genome, also present in four other *L. braziliensis* isolates, is single stranded RNA based on its sensitivity to RNase and alkali but not DNase. This RNA is linear, terminating in 5' phosphate and 3' hydroxyl groups based on end labelling experiments. Particles that are 32nm in diameter were isolated from cytoplasm in the 130S fraction of sucrose density gradients which contains the 6kb RNA. The physical characteristics and RNA content of the particles were examined under various conditions. The stability of the particle under various salt concentrations, ionic conditions, and pH were examined. Portions of two 6kb viral RNAs have been cloned as cDNAs and sequenced. No homology to other viral RNAs has been detected. The 6kb RNA from two isolates are not identical. RNA from various isolates is being examined with cloned probes to determine the extent of homology among the 6kb nucleic acids. Also see Tarr, P.I., R.F. Aline Jr., B.L. Smiley, J. Scholler, J. Keithly & K. Stuart. 1988. PNAS (in press).

O 230 THE SPLICED LEADER SEQUENCE OF *CAENORHABDITIS ELEGANS* OCCURS WITHIN MANY GENES OF *ONCHOCERCA VOLVULUS*, Wenlin Zeng and John E. Donelson, Department of Biochemistry,University of Iowa, Iowa City, IA 52242

Genomic DNAs of the parasitic nematodes *Onchocerca volvulus* and *Dirofilariae immitis* and a cDNA library of *O. volvulus* were examined for the presence of the 22 nucleotide spliced leader sequence found in some, but not all, mRNAs of the free living nematode *Caenorhabditis elegans*. Similar copies of this leader RNA gene were found to be linked to the repetitive 5S rRNA genes in *O. volvulus* and *D. immitis*, as in *C. elegans*. Sequence comparison of the 5S rRNA genes and the leader RNA genes in *O. volvulus* and *D. immitis* revealed a high degree of heterogeneity in the 5S rRNA genes, whereas the leader RNA genes are more conserved. In addition to the genes linked to the 5S rRNA genes, the 22 nucleotide leader sequence is also encoded as a conserved 25-mer at over 50 genomic sites and occurs within many polyA+ transcripts in *O. volvulus*. Southern blots show a similar scattered distribution of this sequence in two other parasitic nematodes, *Brugia malayi* and *D. immitis*, but not in the free living nematode, *C. elegans*, where it occurs mainly on a repetitive DNA element. Sequence determination of four different cDNAs of *O. volvulus* containing this 22-mer and one of their corresponding genomic clones indicated this sequence is not trans-spliced onto the RNAs, but is encoded within the genes. The conservation of this 22 nucleotide sequence in both parasitic and free living nematodes argues that it serves a specific biological function for these organisms.

Evolution; Epidemiology

O 300 AN RFLP THAT RELIABLY PREDICTS DRUG RESISTANCE IN SCHISTOSOMA MANSONI RESULTS FROM THE INTEGRATION OF A MOBILE ELEMENT INTO A rRNA GENE. P. Brindley[1], F. Lewis[2], T. McCutchan[1], E. Bueding[3], & A. Sher[1], [1]LPD, NIAID, NIH, Bethesda, [2]Biomedical Research Institute, Rockville & [3]Johns Hopkins University, Baltimore MD. Ribosomal gene probes were used to investigate the genetic basis of resistance to hycanthone (HC) in Schistosoma mansoni in a model in which resistance is induced by exposing immature worms in mice to the drug. Probe pSM389 (which contains part of the small rRNA gene plus non-transcribed spacer) detected RFLPs in Southern blotted DNA of HC-resistant parasites restricted with several different enzymes. The most reliable marker of drug resistance was a 3.6 kb Bam H1 RFLP which appeared consistently in individuals and populations of resistant worms from different stocks and generations but was absent from the drug sensitive parent schistosomes as well as other HC sensitive strains. Since the RFLP was not observed in over 100 different drug sensitive parents, it clearly derives from an induced genetic change rather than from selection of preexisting drug resistant worms. Structural analysis of the cloned 3.6 kb fragment hybridizing with pSM389 indicates that the RFLP arises from the integration of a mobile element of 0.5 kb length into the 5' terminus of one or several small rRNA genes. These results suggest that drug resistance in schistosomes is associated with and may result from genomic rearrangement.

O 301 FIELD TESTING OF A MONOCLONAL PROBE SPECIFIC FOR INFECTIVE LARVAE OF HUMAN BRUGIAN PARASITES. Clotilde K.S. Carlow*, A. Suwita #, Z. Bahang +, Purnomo #, F. Partono # and Mario Philipp*. *New England Biolabs, Beverly, MA 01915, # University of Indonesia, Jakarta, Indonesia, + Ministry of Health, Jakarta, Indonesia.

Epidemiological surveys and assessement of control programmes for Brugian filariasis require the identification of natural vectors and determination of their transmission potential. This necessitates both the distinction of human Brugian parasites from the various animal filariae transmitted by the same mosquito and the evaluation of infective stages present. Neither task can be performed using morphological criteria. We have previously described the species- and stage-specific properties of a monoclonal antibody reactive with the surface of *Brugia malayi* and *B.timori* infective stage larvae (1). A simple monoclonal antibody-based immunoenzyme assay was developed (1) and we have currently investigated its applicability to field studies in an endemic area. Large numbers of wild mosquitoes consisting of 28 species belonging to 6 genera were collected in South Kalimantan, Borneo, Indonesia and examined in the field for the presence of human Brugian filariae. We evaluated the specificity and sensitivity of the assay using natural vectors and wild type *B.malayi*, *B.timori* and *B.pahangi* infective larvae. Specificity was 100% and sensitivity was consistently higher than 90%.

(1) Carlow, C.K.S. et al. (1987) Proc. Natl. Acad. Sci. 84, 6914.

O 302 EVOLUTION OF THE AMOEBOFLAGELLATE GENUS NAEGLERIA AS REVEALED BY ANALYSIS OF ITS CIRCULAR RIBOSOMAL RNA GENES, C. Graham Clark, George A.M. Cross, Johan F. De Jonckheere+, Laboratory of Molecular Parasitology, The Rockefeller University, New York NY 10021, USA and +Departement Microbiologie, Instituut voor Hygiëne en Epidemiologie, B-1050 Brussels, Belgium. Species in the protozoan genus *Naegleria* are ubiquitous, free-living, bacteriovorous, soil amoebae that have the unusual ability to transform under stress conditions into flagellates. In addition, several species are known to be facultatively pathogenic for mammals, with *N. fowleri* being the etiological agent of primary amoebic meningo-encephalitis in humans. We have discovered that the ribosomal RNA genes (rDNA) of *Naegleria* and related genera are extrachromosomal and circular with one rRNA transcription unit per plasmid. They are present in multiple copies per cell, making up 17% of the total DNA in the strain most closely studied (*N. gruberi* NEG-M), but no chromosomally integrated copy can be detected. Classification of isolates within the genus *Naegleria* is not trivial since all species are morphologically indistinguishable. rDNA plasmid restriction patterns were therefore evaluated as a classification tool. Complete rDNA units have been cloned from two species and rDNA restriction maps of one strain from each *Naegleria* species have been constructed and the interspecific relationships determined. Characteristic species patterns were indeed obtained and the three pathogenic species are not closely related one to the other. Intraspecific variation has also been evaluated and, while the integrity of most species is indeed supported, *N. gruberi* appears to be a polyphyletic assemblage of strains.

O 303 RESTRICTION FRAGMENT LENGTH POLYMORPHISMS CAN BE USED TO IDENTIFY EIMERIA SPECIES. J Ellis, J Bumstead, L Clarke, F Tomley. AFRC Institute for Animal Health, Houghton Laboratory, Houghton, Huntingdon, Cambs. Cloned DNA sequences, coding for the large and small subunit rRNAs and 5S rRNA, have been isolated from the genome of Eimeria tenella and partially characterised. DNA hybridisation studies have demonstrated that there are very few genes coding for the rRNAs, although the 5S rRNA gene is represented by a separate, tandemly repeated sequence of very high copy number. It has been demonstrated that individual Eimeria species can be identified by virtue of their DNA fingerprints using these DNA probes.

O 304 A MITOCHONDRIAL MEMBER OF THE HSP70 FAMILY, David M. Engman, Elizabeth A. Dragon* & John E. Donelson, Department of Biochemistry and Program in Genetics, University of Iowa, Iowa City, IA 52242 and *Codon, S. San Francisco, CA 94080

A gene isolated from the protozoan parasite Trypanosoma cruzi encodes a protein of relative molecular mass 71,000 (71K) that is homologous with the 70K heat shock and glucose-regulated proteins (hsp70s). A comparison of p71 with these proteins revealed that it is most similar to the DnaK protein of E. coli. p71 is, however, unique among eukaryotic hsp70s in that it is located in the mitochondrion. Within the trypanosome mitochondrion, the protein associates with the kinetoplast, a sub-organellar structure that lies within the mitochondrial matrix and houses the kinetoplast DNA (kDNA), the unusual mitochondrial DNA that distinguishes this order of protozoan. p71 possesses an N-terminal leader of about 24 amino acids that has the features of a mitochondrial signal sequence and presumably accounts for its intracellular location. In contrast to the mRNA level of the trypanosome hsp70, which increases in response to heat shock, the mRNA level of p71 decreases upon heat shock. The similarity of p71 to E. coli DnaK, combined with its association with kDNA, suggests that p71 participates in DNA replication in mitochondria in a manner analogous to that of DnaK in E. coli. These findings have implications for the molecular evolution of the eukaryotic hsp70 multigene family.

The results of a molecular genetic and immunological analysis of T. cruzi p71 and hsp70 will also be presented.

O 305 APPLICATION OF MOLECULAR KARYOTYPE ANALYSIS TO THE EPIDEMIOLOGY OF RECURRENT CUTANEOUS LEISHMANIASIS FROM THE PACIFIC COAST REGION OF COLOMBIA, S.H. Giannini[1] and N.G. Saravia[2], Department of Medicine and Center for Vaccine Development, University of Maryland School of Medicine, Baltimore MD 21201 and [2]Centro Internacional de Investigaciones Medicas, CIDEIM, Colciencias, Cali, COLOMBIA

Twenty-five Leishmania braziliensis panamensis stocks isolated from primary (1°) or recurrent (2°) lesions of 12 patients with recurrent cutaneous leishmaniasis were karyotyped by transverse alternating field electrophoresis and by pulsed field gel electrophoresis with a hexagonal electrode. Several size conserved chromosome bands were identified for this collection. Probes for pR4, heat shock protein 70, glyceraldehyde phosphate dehydrogenase, and α,β-tubulin preferentially hybridized to size-conserved bands in all stocks tested. Thus size-homologous chromosomes may also share sequence homology, and a mechanism may operate to conserve some linkage groups. The presence of hypervariable chromosomes, found in some stocks and not others, resulted in a unique molecular karyotype for isolates from almost every patient. In 4/12 of the patients the 1° and 2° isolates differed, probably indicating reinfection. In the remaining patients, the 1° and 2° isolates were indistinguishable, likely indicating a reactivation of the original infection, although reinfection with the same stock cannot be ruled out. DNA was purified from a chromosome of ~388 kb found in some stocks but not in others. When nick translated, the hypervariable DNA hybridized to the size-homologous bands (where they were present), and also to bands of different size in the other stocks as well as in the homologous stock. Thus the molecular karyotype may allow identification of "hot spots" in the genome. (Supported by USPHS Grant AI23956)

O 306 IDENTIFICATION AND STUDY OF A NEW SPECIES OF LEISHMANIA USING MONOCLONAL ANTIBODIES, Catherine A. Hanham, Jeffery J. Shaw, Ralph Lainson and Diane McMahon-Pratt, The London School of Hygiene and Tropical Medicine, Keppel Street, London, England.

Species-specific monoclonal antibodies provide useful tools for the rapid identification of Leishmania by radioimmunoassay and indirect immunofluorescent tests. Investigations into the epidemiology of leishmaniasis in South America are, however, often hampered, since many Leishmania isolates cannot be positively identified using currently available monoclonal antibodies. Here we describe the development of new monoclonal antibodies which recognise one such parasite, Leishmania (Viannia) lainsoni, which has recently been isolated from patients with cutaneous leishmaniasis in the Amazon region of Brazil. Three monoclonal antibodies were produced which all reacted with the same high molecular weight molecule present in membrane preparations of L. (V.) lainsoni strains, as evidenced by Western blot analysis. Differences in specificity between these monoclonal antibodies were seen, however, using radioimmunoassay, and to a lesser extent, indirect immunofluorescence. One possible explanation for these results could be that the monoclonal antibodies recognise epitopes on a single molecule which shows conformational differences between various strains of the parasite. Our results show antigenic heterogeneity within species of Leishmania which have not previously been detected by isoenzyme electrophoresis.

O 307 SOME EXPRESSION SITE ASSOCIATED GENES MAY BE EVOLUTIONARILY RELATED TO VSG GENES, Maurine R. Hobbs and John C. Boothroyd, Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305. A family of *Trypanosoma brucei* genes (BS1) have been identified that are transcribed in the bloodstream form but not the insect procyclic form of the parasite's life cycle. These genes (~15-20/cell) are distributed throughout the genome on all chromosome size classes except the smallest "mini-chromosomes". They are present and transcribed in several different *T. brucei* variant types as well as in *T. gambiense*. Although they share no sequence similarity to expression site associated gene 1 (ESAG1) (Cully et. al., 1985, Cell 42, p.173-182) or ESAGs 2 and 3 (Alexandre et. al.,1988, Mol. Cell. Biol. 8, p.2367-2378), they are >85% identical with the genes identified at the 5' end of the putative 221 expression site (Kooter et. al.,1987, Cell 51, p.261-272; and personal communication). Together these results suggest that the gene under study is another ESAG.

The translated protein sequence shows significant homology between the BS1 genes and a known VSG gene (Lenardo et. al., 1984, P.N.A.S. 81, p.6642-6646), suggesting evolutionary relatedness. There are three interesting hypotheses that could explain these results. The first is that the degree of homology between BS1s and VSGs is an indication of the frequency of recombinational events in the expression site and/or the result of convergent evolution. The second is that ESAGs are present in the expression site because they are remnants of earlier VSGs. The third and perhaps most interesting is that ESAGs may represent the evolutionary origins of VSGs from non-variant specific protein genes.

O 308 RIBOSOMAL RNA SEQUENCING SHOWS THE SUBPHYLUM SARCODINA TO BE POLYPHYLETIC, Alan M. Johnson, Susi Illana and *Peter R. Baverstock, Department of Clinical Microbiology, Flinders Medical Centre, Bedford Park, 5042 *Evolutionary Biology Unit, South Australian Museum, Adelaide, 5000, South Australia. A rapid RNA sequencing technique was used to partially sequence the small subunit ribosomal RNA of four species (N. fowleri, N. lovaniensis, N. australiensis, N. gruberi) of the amoeboid genus Naegleria. The extent of nucleotide sequence divergence between the two most divergent species (N. gruberi, N. australiensis) was roughly similar to that found between mammals and frogs. However, the pattern of variation among the Naegleria species was quite different from that found for those species of tetrapods characterised to date. A phylogenetic analysis of the consensus Naegleria sequence showed that Naegleria was not monophyletic with either Acanthamoeba castellanii or Dictyostelium discoideum, two other amoebae for which sequences were available. It was shown that the semi-conserved regions of the srRNA molecule evolve in a clock-like fashion, and that the clock is time-dependent rather than generation-dependent.

O 309 A CONSERVED EXTRACHROMOSOMAL DNA ELEMENT FROM *PLASMODIUM GALLINACEUM* CONTAINING THE GENE FOR CYTOCHROME B,
Jeffrey T. Joseph*, Susan M. Aldritt† and Dyann F. Wirth*†, *Division of Medical Sciences, Harvard Medical School, and †Department of Tropical Public Health, Harvard School of Public Health, Boston, MA 02115.

We have isolated and characterized a six kilobase moderately repeated circularly permuted DNA element from *Plasmodium gallinaceum*. The element encodes at least five RNA's which are transcribed off both DNA strands. Identical, highly conserved elements are found in all *Plasmodium* species tested and similar elements are found in other members of the Apicomplexa phylum. This piece of DNA is extrachromosomal, as demonstrated by its migration patterns both in sucrose gradients and on chromosome gels. It can be purified from chromosomal DNA on Hoechst dye - CsCl gradients. The element is linear and tandemly repeated, as determined by its partial restriction pattern, its sensitivity to exonuclease III, and its appearance in the electron microscope. The element also contains a gene homologous to cytochrome b, based on sequence similarity and hydropathy plots.

From these results, we postulate the element is a mitochondrial episome conserved in the Apicomplexa phylum .

O 310 IMMUNODIAGNOSIS OF NEUROCYSTICERCOSIS

B.V.Ravi Kumar, V.Suryanarayana, Achyut M.Sinha, Charu Ramakrishnan, S.D.Roopashri, ASTRA RESEARCH CENTRE INDIA, BANGALORE 560 003, INDIA.

Neurocysticercosis caused by the infestation of human brain by the larvae of tapeworm Taenia solium mimics many infections and neoplastic conditions of CNS and needs a specific and sensitive diagnosis. In order to characterise the antigens the larvae were maintained in vitro. Secretory/excretory antigens that appeared in the medium conferred 90% sensitivity and 90% specificity to an ELISA, detecting antibodies in cereberospinal fluids (CSF) of patients. Polypeptides of mol wt 66000 and 38000 and Con A and wheal germ agglutinin binding glycoproteins of 97000 and 50000 were most frequently recognised by the antibodies in CSFs. Screening a cDNA library with an oligonucleotide probe derived from a cDNA clone of 38 KDa antigen. Monoclonal antibodies for some of these polypeptides were obtained to screen for expression of the antigens in mammalian cells.

O 311 CHARACTERIZATION OF A ≈400 NT INTRON IN THE 18S rRNA GENE(S) OF PNEUMOCYSTIS CARINII. Hua Lin and Gregory A. Buck. Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 23298. The 18S rRNA of the AIDS pathogen P. carinii was directly sequenced using reverse transcriptase and Sanger chain termination methodology. The sequence obtained was similar to a recently published sequence derived from a DNA clone (1) except for the absence of a putative ≈400 nt intron near the 3' terminus of the gene. Comparison of the P. carinii sequence with the 18S rRNA sequences of other organisms suggests that P. carinii is more closely related to fungi than to protozoa. To verifiy the presence of the intron in the P. carinii DNA, synthetic oligonucleotides homologous to the sequences flanking the putative intron were used in Polymerase Chain Reactions to directly amplify the corresponding genomic fragment. The resultant amplified DNA reflected the predicted size of the intron. No other amplified bands were observed, suggesting that all copies of the P. carinii 18S gene bear the intron. The amplified DNA was purified and directly sequenced and the sequence verified the presence of a ≈400 nt putative Group I intron.

1. Edman et al. Ribosomal RNA sequence shows Pneumocystis carinii to be a member of the Fungi. Nature 334: 519-522 (1988).
2. Saiki et al. Primer-Directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487-491 (1988).

O 312 VARIATION IN THE PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN GENE: IMPLICATIONS FOR VACCINE DEVELOPMENT. Michael J.Lockyer, Department of Molecular Biology, Wellcome Biotech, Langley Court, Beckenham, Kent, BR3 3BS.

The circumsporozoite protein (CSP) is the major candidate for a sporozoite malaria vaccine. While the overall structure and organization of the P.falciparum CSP gene is similar in different lines, it is becoming clear that the potential for variation is greater than was first apparent. The DNA sequence of the CSP genes from two cloned Thai lines, T9-98 and T9-101 has been determined - the sequence of the T9-98 gene reveals a 57bp deletion in the 5' coding region at a site where a 30bp insertion is found in some strains, and two previously undescribed amino acid substitutions one in each of two areas of the 3' coding region shown to be human immunodominant T-cell sites. The central immunodominant repeat region of the T9-101 CSP gene shows a novel interspersion pattern of major and minor repeats. Amino acid substitutions have now been mapped to 13 different positions in the CSP gene sequence with no two CSP genes showing the same substitution at every position. The coincidence of these substitutions with putative functional regions and immunodominant T-cell epitopes is disturbing in its implications for development of an effective sporozoite vaccine.

O 313 REPETITIVE SEQUENCES IN THE SMALL DNA-CHROMOSOME OF L.BRAZILIENSIS (M2903).
Alexis Mendoza, Salih Eresh and Douglas Barker. Molteno Labs., Department of Pathology, University of Cambridge, Cambridge CB2 1QP, UK.

The presence of multiple copy families of genes and non-coding sequences have been demonstrated in most eukaryotic genomes. In some parasitic diseases such sequences can be used for identifi cation and distinguishing between closely related organisms. We have resolved by PFE chromosome sized DNA molecules in stocks of the New World Leishmania. The smallest chromosome, identified only in one braziliensis stock, stains intensely with ethidium bromide which possibly indicates the presence of multicopy sequences with specificity at species level. When this chromosome was used as a probe to hybridise a blot of a PFE gel, homology was observed to distinct chromosomal DNA bands in braziliensis stocks only, suggesting the presence of some sequences which show variation in chromosomal location between subspecies and specificity at species level. Low stringency hybridisation of this probe to genomic DNA, from different Leishmania isolates of mexicana and braziliensis complexes, digested with various restriction enzymes, detected multiple DNA fragments in the homologous DNA and in DNA of other braziliensis stocks. A weak cross-hybridisation to DNA from L. mexicana occurs, which was eliminated after high stringency washing. Thus, the results suggest the presence in the small DNA-chromosome of different repetitive DNA, some of them species-specific, with polymorphism in length and copy number. Cloning and characterization of these sequences are in progress.

O 314 IDENTIFICATION AND SEQUENCE DATA OF TRANSCRIPTS OF THE CD1 RELATED ELEMENTS OF LEISHMANIA,
N. Gajendran, J.-Cl. Dujardin, S. Muyldermans, D. Le Ray, R. Hamers, Vrije Universiteit Brussel, Instituut voor Moleculaire Biologie, Paardenstraat 65, 1640 Sint-Genesius-Rode, Belgium

We have recently identified small circular and linear DNA elements in Leishmania (N.Gajendran, et al., R. Hamers, et al.). These elements could possess transposon like properties and can be partially integrated into other chromosomes. Transcripts from these elements have been observed by probing Northern blots with cDNA clones. These clones were picked up from a cDNA library using CD1 (circular DNA1) as a probe. The observed transcripts appear to be in the size range from about 3.9Kb and larger. Some of the circular and linear elements seem to have some transcripts in common. Patterns from restriction enzyme digests and subsequent hybridization to Southern blots also indicate differences within the circular and between the circular and linear elements. The elements have been found in both New and Old World Leishmania.

[1]Gajenran, N., Dujardin, J.-CL., Le Ray, D., Matthyssens, G. and Hamers, R., 1987. Abnormally migrating chromosome identifies Leishmania donovani populations, in: "Leishmaniasis: the current status and new strategies for control", series A. volume 163, Plenum Press.

[2]Hamers, R., Gajendran, N., Dujardin, J.-Cl. and Stuart, K. Circular and linear forms of small nucleic acids in Leishmania, in: "Leishmaniasis: the current status and new strategies for control", series A, volume 163, Plenum Press.

O 315 CONSERVED AND POLYMORPHIC T CELL EPITOPES IN THE P.FALCIPARUM CS PROTEIN,
J.R.L. Pink, P. Caspers, M. Guttinger, J. Kilgus, H. Matile, P. Romagnoli, D. Stüber and F. Sinigaglia, Central Research Unit, F. Hoffmann-La Roche & Co. Ltd., 4002 Basle, Switzerland.

We synthesised peptides corresponding to regions of the CS protein which were predicted to contain T cell epitopes and tested whether they stimulated proliferation of blood cells from malaria-exposed or non-exposed donors. One peptide, CS.T3 (CS protein residues 378-398 with Cys replaced by Ala) corresponds to a conserved region of the CS protein and can be recognised by human $CD4^+$ T cell clones in association with many different MHC class II molecules (Sinigaglia et al., Nature, in press). CS.T3-specific clones could recognise parasite-derived CS protein. Mice preimmunised with CS.T3 had increased anti-parasite responses following immunisation with sporozoites, suggesting that inclusion of CS.T3 sequences in a vaccine would allow boosting of antibody responses following natural infection. A second peptide, CS.T2 (CS protein 325-341) includes residues which vary in different P. falciparum isolates, e.g., position 339 (Lys or Gln). Human T cell clones recognised the Lys339 peptide in association with HLA-DR5, but did not respond to the Gln339 peptide or the NF54 isolate CS protein, which has been completely sequenced and contains Gln at position 339. Both peptides were shown by an in vitro competition assay (Kilgus et al., PNAS, in press) to bind to DR5, suggesting that differential binding of the parasite-derived polymorphic peptides to DR5 does not contribute to maintenance of the polymorphism by immune selection.

O 316 DEVELOPMENT OF A DNA ASSAY FOR THE IDENTIFICATION AND QUANTITATION OF MICROFILARIAE IN BLOOD SAMPLES USING BIOTINYLATED OLIGONUCLEOTIDE PROBES, C. Poole*, S. Williams+*, T. Supali#, D. Landry* and L. McReynolds*. *New England Biolabs, Beverly, Mass. +Dept. of Biological Sciences, Smith College, Northampton, Mass. #Dept. of Parasitology, University of Indonesia, Jakarta, Indonesia.

Due to difficulties in detection and species identification of lymphatic filarial parasites in human, animal and mosquito hosts, accurate epidemiological data are very difficult to obtain. We have designed a parasite detection assay, using labeled DNA probes that can give both quantitative information (number of parasites present) and qualitative information (species of parasite present). The DNA probes used in the assay were derived from a DNA repeat family, the Hha I repeat family, common to both the filarial parasites, Brugia malayi and B. pahangi. In addition, we have determined that adding EDTA to infected blood samples to a final concentration of 100mM protects parasite DNA from degradation for up to 28 days at 37°C. This is a simple and efficient way of preserving parasite DNA until blood samples, collected in the field, can be analyzed.

The design of the assay is based upon filtration of infected blood samples through nitrocellulose membranes without prior purification of the parasite DNA. When the assay is used with radioactive DNA probes, the number of parasites in a blood sample can be assessed by simply counting individual spots on autoradiograms. However, problems with using radioactivity in the field have prompted us to develop non-radioactive probes. A series of oligonucleotide DNA probes have been synthesized each consisting of a different number of biotinylated nucleotides. The greatest sensitivity has been acheived with a probe containing 45 biotins attached to the 5' end of the molecule. Using this probe, we have been able to detect as little as 50 picograms of parasite DNA on slot-blots (the amount of DNA in approximately half a microfilariae). Presently, we are assessing the species-specificity and sensitivity of the assay when used with the biotinylated probes.

O 317 A HISTOLYTIC SERINE PROTEASE FROM A PARASITIC NEMATODE LARVA IS SIMILAR TO MAMMALIAN TRYPSIN, Judy A. Sakanari and James H. McKerrow, Department of Pathology, University of California, San Francisco, CA 94143

Ingestion of larval nematodes belonging to the family Anisakidae can cause the human disease known as anisakiasis. After ingestion, worms such as *Anisakis*, can be invasive, penetrating host stomach and intestinal tissues. Because these worms are capable of penetrating human tissues, we hypothesized that secreted proteases may be involved in degradation of host tissues. Using an *in vitro* model of extracellular matrix (ECM), we found that live *Anisakis* larvae could degrade approximately 25% of the ECM in 24 hours. Further characterization of the secreted proteases revealed that there are two classes of proteases: a metalloprotease and a serine protease. The metalloprotease is an aminopeptidase, and the serine protease has trypsin-like activity with synthetic peptide substrates. *Anisakis* extract contains a 25-kD protease that is recognized by rabbit anti-rat trypsin antibody on Western blots. A serine protease gene fragment has been cloned and is 67% identical to rat trypsin II at the nucleic acid level. Sequence analysis also showed that the catalytic triad of the active site of serine proteases was conserved in this parasitic nematode.

O 318 SENSITIVE DETECTION AND SCHIZODEME CLASSIFICATION OF TRYPANOSOMA CRUZI BY AMPLIFICATION OF KINETOPLAST MINICIRCLE DNA SEQUENCES, Nancy R. Sturm, Herbert Avila, Antonio Goncalves, Wim Degrave, Carlos Morel and Larry Simpson, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024, and Department of Molecular Biology, Oswaldo Cruz Institute, Rio de Janeiro, Brazil

Two overlapping fragments (83 and 122 bp) from the conserved 120 bp minirepeat regions of the minicircle DNA and one fragment (330 bp) covering all the adjacent variable regions were amplified by the polymerase chain reaction. The minimal amount of minicircle DNA required to detect a product by hybridization was 0.015 fg, or approximately 10 molecules or 0.1% of the minicircle DNA component of a single cell. The amplification was T. cruzi strain-independent and did not occur with kDNA from several other kinetoplastids. The presence of a several billion fold excess of human DNA has no effect on the amplification process. A simple lysis and deproteinization method was employed to recover kDNA from total blood, using human DNA as a carrier for ethanol precipitation of parasite DNA. Schizodeme analysis was performed on 49 stocks, strains and clones of T. cruzi by acrylamide gradient gel electrophoresis of both total kDNA and the amplified 330 bp variable region minicircle DNA after digestion with several restriction enzymes. These 49 samples represented stocks of T. cruzi isolated from humans, animals and insects from several geographical areas, including Brazil, Venezuela, Colombia and Costa Rica. There was a good correspondence between the schizodeme groupings determined by analysis of total kDNA and amplified variable region DNA. These results suggest that detection of T. cruzi and schizodeme analysis can be taken to the single cell level, thus avoiding the inherent problem of selection involved in growth of parasites to sufficient numbers for analysis by standard methods.

O 319 CONSERVATION OF MINICIRCLE SEQUENCES AND STRUCTURAL FEATURES AMONG KINETOPLASTID PROTOZOA FROM DIFFERENT GENERA.

Otavio Thiemann, Ulisses Lopes, Carlos Morel, Larry Simpson* and Wim Degrave Department of Biochemistry and Molecular Biology, Fiocruz, Rio de Janeiro, 21045, Brasil, and *Department of Biology, University of California, Los Angeles, CA.

By computer alignment, consensus sequences were constructed for the conserved regions of the kinetoplast minicircle DNA molecules from T.cruzi, T.lewisi, T.brucei, T.equiperdum, T.congolense, C.fasciculata, L.tarentolae, L.aethiopica, L.mexicana and L.chagasi. The length of the alignment is dependent upon the species and contains in all cases the 12mer replication origin sequence. It was possible to generate overall alignments of each of the consensus sequences against each other and to construct dendograms using evolutionary and standard parsimony. Conservation of other structural features, such as local sequence composition, bend position and structure was analysed.

This research was supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), CNPq and by NIH research grant to L.S.

O 320 TYPING OF TRYPANOSOMA CRUZI BY NUCLEAR DNA PROBES, Yara M. Traub-Csekö and Alice M. Takeuchi, Departamento de Bioquímica e Biologia Molecular Fundação Oswaldo Cruz, Av. Brasil 4365, Rio de Janeiro, RJ, Brazil.

Trypanosoma cruzi, the causative agent of Chagas' disease, shows heterogeneity in several biological and biochemical properties and causes different clinical manifestations. There is a strong interest to establish reliable ways of classifying these organisms. Monoclonal antibodies, zymodeme and schizodeme analyses have been used with this purpose. Recently cloned nuclear DNA fragments have been used with success to differentiate isolates, strains and species of various parasites. Our aim is to develop probes to be used as genetic markers to type T. cruzi strains and isolates. We have constructed a library of T. cruzi (Y) DNA in pBR322 and tested various clones initially on well characterized laboratory strains, namely CL, Colombiana, DM28, F and Y. Some of the tested probes gave rise to a very simple and conserved band pattern, others gave different degrees of band heterogeneity among the strains. We constructed a dendogram based on our results and it fits some published zymodeme results. We are presently testing other clones and using them to probe field isolates from different geographical regions.

CNPq, INSERM

O 321 ISOLATION OF A HIGHLY REPEATED W-CHROMOSOME SCHISTOSOMA MANSONI DNA PROBE AND ITS USE IN AN ASSAY OF CERCARIAL SEX. Philippa Webster, Tag E. Mansour and David Bieber. Department of Pharmacology, Stanford University Medical Center, Stanford, CA 94305.

In Schistosoma mansoni, the female is the heterogametic sex; the sex chromosomes in the female and male are designated ZW and ZZ, respectively. The sexes are visually indistinguishable in the cercarial stage. A single miracidium can asexually generate thousands of identical cercariae; it is useful to be able to determine the sex of such a clonal population of cercariae both for studying unisexual infections in mice and for establishing genetic crosses. We have cloned and sequenced a 482 bp fragment of female-specific S. mansoni genomic DNA, W1, which is species-specific and present in more than 5,000 copies per female genome. W1 is not transcribed and is likely to be part of the constitutive heterochromatin of the W chromosome. We have developed a rapid and reliable dot-blot assay for determining the sex of S. mansoni cercariae using W1 as a probe. The approach used for cloning W1 may be useful for isolating repeated DNA fragments of the W or Y chromosomes in the heterogametic sex of other species. (Supported by grants from the MacArthur Foundation and NIH.)

O 322 HIGHLY REPEATED DNA SEQUENCES AS SENSITIVE PROBES FOR THE SPECIES SPECIFIC DETECTION OF BRUGIA AND WUCHERERIA IN HUMAN BLOOD SAMPLES FROM INDONESIA, Steven A. Williams[1,2], Daniel J. Freedman[1,2], Janet Glover[1], Shalina Mahajan[1], Paskasari Permana[1], Anita Myer[2], Larry A. McReynolds[3], Catherine B. Poole[3], Taniawati Supali[1,4], Purnomo[4] and Felix Partono[4]; [1]Dept. of Biological Sciences, Smith College, Northampton, MA 01063, [2]Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, MA 01003, [3]New England Biolabs, Beverly, MA 01915, [4]University of Indonesia, Jakarta, Indonesia. We have cloned and characterized highly repeated DNA sequences from several species and many geographic isolates of filarial parasites from Indonesia. These repeats have been used to design DNA probes for sensitive and species-specific detection of filaria in human and animal blood and in mosquito squashes. These DNA probes can be labeled radioactively or non-radioactively for use in field studies. Members of the Hha I repeat family have been cloned and sequenced from B.malayi (six isolates), B.pahangi and B.timori. B.malayi and B.pahangi repeats show 11% sequence divergence, and DNA probes that can distinguish these two species have been devised. These probes were tested in a WHO sponsored field study in S.Kalimantan, Indonesia. The results of this study demonstrated that the DNA probes were as sensitive and species-specific as the traditional method of counting microfilariae in stained blood smears. The B.malayi probe detects all six isolates of B.malayi including one originally from Malaysia and five from Indonesia. The five from Indonesia are all from different islands and include periodic, sub-periodic, zoophilic and anthropophilic isolates. The B.malayi probe also detects B.timori efficiently. The genetic variation seen between the different geographic isolates of B.malayi and B.timori is small enough that the malayi probe detects all of them efficiently. Repeated DNA sequences have also been cloned and characterized from W.bancrofti from Indonesia. When used as DNA probes, these repeats have also proven to be sensitive and species-specific.

O 323 MITOCHONDRIAL DNA OF Plasmodium falciparum: ORGANIZATION AND SEQUENCE OF THE RIBOSOMAL RNAs. Robert J. M. Wilson, Malcolm J. Gardner and Donald H. Williamson. National Institute for Medical Research, Mill Hill, London, NW7 1AA, U.K.

The mitochondrial (mt) DNA of P. falciparum consists of a covalently closed circular molecule with an average contour length of about 11 μm. Because the contribution of the mitochondrion to the parasite's metabolism is not understood, knowledge of mitochondrial genes and their expression should provide insight into mitochondrial function and delineate targets for drug design. Sequence analysis of cloned mt DNA fragments has identified small and large subunit rRNA genes on different strands. There may be more than one copy of each gene. A secondary structure model, derived from the small subunit sequence, shows universally conserved structural elements common to many mt small subunit rRNAs. Preliminary evidence suggests that this gene is expressed in erythrocytic parasites. Our goal is to clone the remaining mitochondrial genes of P. falciparum and to study their expression during various stages of the life cycle.

O 324 AN ELECTROPHORETIC KARYOTYPE FOR PNEUMOCYSTIS CARINII AND IDENTIFICATION OF THE CHROMOSOMES BEARING THE rDNA AND SEVERAL CONSERVED GENES. **Thillanathan Yoganathan and Gregory A. Buck. Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 23298.** Orthogonal field agarose gel electrophoresis of DNA from the AIDS pathogen P. carinii defined ≈12 discrete chromosomes ranging in size from 50-1500 kb. Summation of the estimated sizes of these chromosomes demonstrated that the total complexity of the P. carinii genome is approximately 5-10 megabase pairs. Southern hybridization analyses of these chromosome gels using specific rDNA probes and synthetic oligonucleotides showed that the major ribosomal gene cluster is located on chromosome #7. Similar analyses using heterologous probes for yeast histone H2A showed that the homologous P. carinii histone gene is located on chromosome #5. Other specific gene probes were generated by Polymerase Chain Reaction (1) amplification from purified P. carinii DNA and similarly used to assign the homologous P. carinii genes to the appropriate chromosomes.

1. Saiki et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487-491 (1988).

Gene Expression; Membrane Molecules-I

O 400 A HEAT SHOCK PROTEIN GENE IN GIATDIA LAMBLIA UNRELATED TO HSP70; Aggarwal, Anita, Vidal de la Cruz and Theodore E. Nash, Laboratory of Parasitic Diseases,National Institute of Allergy and Infectious Diseases, NIH, Bethesda,MD.
Heat shock proteins (HSPs) have been reffered as ubiquitous in that they have been found in every organisms investigated, from bacteria to human. They are transiently expressed when the cells are subjected to high temperature or other stressful conditions and their increase in expression may be due to an increase in transcription and prefential translation. A heat shock protein gene was cloned from Giardia lamblia, isolate WB.This gene fragment showed no significant similarity to HSPs from other organisms at nucleotide or amino acid level but was shown to be heat inducible in vivo as determined by an increase in in RNA transcription . DNA sequence analysis revealed a putative heat shock promoter which has consensus conserved sequence similar to the regulatory sequence of the other organisms. The biological function of the heat shock promoter was shown by an increase inbeta-galactosidase enzyme activity in Giardia and yeast transformed with plasmid having synthetic promoter oligonucleotides based on Giardia sequence. This gene fragment was not present in some of the isolates indicating that the gene is not required for the survival of the parasite in unfavourable conditions.

O 401 A DIFFERENTIAL SCREENING SYSTEM TO ANALYZE NEW GENES CODING FOR ANTIGENS OF PLASMODIUM FALCIPARUM. Antonio Alcina and G. Nigel Godson, Department of Biochemistry, New York University Medical Center, 550 First Ave, New York, New York 10016.

We are sequencing and characterizing Lambda-gt11 cloned genes coding for P. falciparum antigens recognized by malaria patient sera from different geographical locales.

Sera from individual African and Indian patients were characterized for inhibition of growth of P. falciparum blood stages in culture. These pool sera were used to isolate 110 Lambda-gt11 clones from cDNA and genomic DNA expression libraries. The clone bank was then screened with sera grouped according to (a) their geographical origin and (b) their growth inhibition.

Clones specific for and common to both Indian and African sera were isolated; clones that specifically reacted with growth inhibiting sera were isolated and clones that cross reacted with a rabbit antihuman RBC membrane protein antibody were isolated. These clones are now being characterized.

O 402 AN EARLY VARIANT SURFACE GLYCOPROTEIN GENE WITH AN INCOMPLETE BASIC COPY, Robert F. Aline Jr. and Kenneth Stuart, Seattle Biomedical Research Institute, 4 Nickerson St., Seattle, WA 98109

The A variant surface glycoprotein (VSG) gene of the IsTaR 1 serodeme of *Trypanosoma brucei* is expressed at high frequency relapse populations. First relapse populations produced from non-A expressing variant antigenic types (VATs) are essentially 100% A-expressors. In all but one VAT of the IsTaR 1 serodeme, the A VSG gene family consists of two A genes: a telomeric gene that can be expressed in situ or used as a basic copy for gene conversion, and an intrachromosomal gene (the A-B gene) whose 3' end differ from that of the telomeric gene. In VAT 5^{A3} the telomeric A gene is lost, leaving only the intrachromosomal A-B gene. Interestingly, the first relapse populations derived from VAT 5^{A3} contained A-expressors, in some cases up to 5% of the population. The A-B gene, therefore, can be classified as an "early" gene in this serodeme even though it is intrachromosomal and can only be expressed through the gene conversion mechanism. Sequence analysis of the 3' end of the A-B gene, however, revealed that the A-B gene is incomplete, lacking the normal sequences found at the 3' end of expressed VSG genes. This suggests that incomplete intrachromosomal VSG genes can also be "early" genes in a serodeme and that something other than a telomeric location may be involved in the preferential expression of certain VSG genes.

O 403 ANAPLASMA MARGINALE: STRICT CONSERVATION OF A NEUTRALIZATION-SENSITIVE EPITOPE IN THE TANDEM REPEAT REGION OF THE POLYMORPHIC SURFACE ANTIGEN, AmF105. D.R. Allred[1], G.H. Palmer[2], S.M. Oberle[1], T.C. McGuire[2], and A.F. Barbet[1]; [1]Dept. Infectious Diseases, Univ. Florida, Gainesville, FL and [2]Dept. Veterinary Microbiology and Pathology, Washington State Univ., Pullman, WA. The immunoprotective surface antigen, AmF105, of Anaplasma marginale is highly polymorphic in different isolates, varying from 70 to 105 kDa in apparent size. We cloned and expressed the AmF105 gene from four isolates using the neutralizing monoclonal antibody, mAb 22B1 (which recognizes AmF105), as a probe. All four alleles were found to be highly homologous over the entire gene, except for the 5' end. A region beginning 28 or 31 bases from the start of the coding sequence contains tandemly-repeated 84mer or 87mer base-pair segments. These repeats are present in 5 basic forms in the four isolates, with no more than two forms present in any one isolate. The size variation is explained entirely by variations from 2 to 8 in the number of repeats present in each isolate. mAb 22B1 was used in immunoassays of synthetic oligopeptides to map the neutralization-sensitive epitope to a 6 amino acid region within the repeats. This epitope is present in two forms, I and II, each of which binds mAb 22B1 equally. Form I or form II epitope is present once in each repeat unit. Expression of recombinant AmF105 and definition of a conserved neutralization-sensitive epitope will enable vaccination trials with recombinant or synthetic analogues of native AmF105.

O 404 COMPONENTS OF A HOST CELL HOMOGENATE BIND TO SPECIFIC ANTIGENS OF EIMERIA SPOROZOITES, P.C. Augustine, U.S. Department of Agriculture, Agricultural Research Service, Protozoan Diseases Laboratory, Beltsville, MD 20705. Sporozoites of Eimeria adenoeides, E. tenella, E. meleagrimitis, and E. acervulina were solubilized in buffer containing sodium dodecylsulfate, electrophoretically separated under reducing conditions, and electroeluted onto nitrocellulose sheets. When the sheets, containing the separated sporozoite bands, were incubated in a homogenate of cultured kidney cells, components of the host cell homogenate bound to several of the sporozoite bands having molecular weights of approximately 23, 40, and 50-60 Kd. Biotinylation of intact sporozoites and incubation in trypsin and chymotrypsin before exposure to the host cell homogenate indicated that the 40 and 50-60 Kd antigens were located within the sporozoite and that the 23 Kd antigen might be located on the sporozoite surface. It is hypothesized that these molecular affinities may reflect sporozoite-host cell interactions that are involved in cellular invasion.

O 405 CLONING AND CHARACTERIZATION OF A cDNA CODING FOR $G_{s\alpha}$ FROM SCHISTOSOMA MANSONI. Max Iltzsch, Mario Zurita, Tag E. Mansour and David Bieber. Department of Pharmacology, Stanford University Medical Center, Stanford, CA 94305.

Biochemical studies in our lab have shown the a G-protein (GTP binding protein) is involved in the serotonin signalling system present in parasitic trematodes. This system is coupled to an effector enzyme, adenylate cyclase, and may be central to the alteration in cyclase activity during development (1). To further characterize this phenomenon, we have isolated a 1400bp cDNA from S. mansoni using the bovine $G_{s\alpha}$ cDNA as a heterologous probe (2). The cDNA which represents the 3'-end of the gene has been sequenced and represents about 960bp of coding region (85% of the size of the mammalian protein) and 450bp of untranslated sequence. The putative S. mansoni protein and the bovine $G_{s\alpha}$ are 68% homologous using exact amino acid homologies and 80% homologous when allowing conservative amino acid substitutions. The genomic organization and the developmental expression profile are presented to correlate the message levels with the change in enzymatic activity seen in somule development. (Supported by grants from the MacArthur Foundation and the NIH).

1. Kasschou, M.R., and Tag E. Mansour. 1982. Nature 296: 66.
2. Harris, B.A., et al. 1985. Science 229: 1274.

O 406 CHARACTERISATION OF THE SECRETORY ACETYLCHOLINESTERASES OF *NIPPOSTRONGYLUS BRASILIENSIS*, C. Clare Blackburn, R. M. Maizels and M. E. Selkirk, Department of Biochemistry, Imperial College, London SW7 2AZ, U.K.)

Many Gastro-Intestinal nematodes actively secrete large quantities of Acetylcholinesterase during the intestinal phase of their lifecycles, although the physiological basis for this phenomenon is unclear. *N. brasiliensis* secretes two Acetylcholinesterase isoforms, the expression pattern and levels of which change over the course of an experimental infection. Specific staining of polyacrylamide gels for Acetylcholinesterase and active site labelling using ^{3}H-DFP have been used to reexamine isoenzyme expression during infection. Switching from predominantly Form A to Form B expression has been found to occur concomitantly with the final moult (L_4-adult), and also to occur during in-vitro culture of immature adult worms. SDS-PAGE analysis has shown isoforms A and B to be monomers of M_r 74KD and 40KD repectively and 2D electrophoresis shows the 40KD isoform to have a p.I. of 4.5 - 5.0. Enzyme purified from preparative PAGE has been used a) to generate partial peptide maps of the two isoforms, b) to deduce their structural relationship via amino acid sequencing, and c) to raise polyclonal anti-AChE for use in *λ gt 11* cloning.

O 407 MOLECULAR CHARACTERISATION OF ANTIGEN GENES Of *ONCHOCERCA VOLVULUS* ISOLATED BY DIFFERENTIAL SCREENING OF A cDNA LIBRARY. Janett E. Bradley, Rudolf Helm, Marc Karam, and Richard M. Maizels. Department of Pure and Applied Biology, Imperial College, Prince Consort Road, London SW7 2BB.

An *O. volvulus* cDNA library (provided by Dr J. Donelson, Univ. Iowa) was screened with pooled onchocerciasis sera. Differential screening with a pool of *W. bancrofti* sera from Papua New Guinea (provided by Dr K. Forsyth PNG Inst. Med. Res.) resulted in a panel of clones only recognised by tl anti- *O. volvulus* sera. These clones were screened with individual sera and 22 were not recognised by any individual with bancroftian filariasis. Several clones were specifically recognised by greater than 90% of the individual *O.volvulus* sera tested. A set of these clones have been subcloned into the plasmid expression vector PNGS 8+ and the fusion proteins purified and used in ELISA. Anti fusion protein sera has been produced and used to characterise the native antigens encoded by these clones. They were all found to be of low molecular weight, one clone encoding a 20K surface molecule of adult worms, and another encoding a 16K microfilarial specific protein.

O 408 EXPRESSION IN *Escherichia coli* OF GENES ENCODING THE MAJOR SURFACE GLYCOPROTEIN FROM *Leishmania*. Linda L. Button and W. Robert McMaster. Department of Medical Genetics, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The major surface glycoprotein of *Leishmania*, gp63, is being developed as a recombinant vaccine against *Leishmaniasis*. Characterization of a cloned gp63 gene from *Leishmania major* indicates that gp63 is synthesized as a precursor protein, containing both a 39-aa signal peptide and a 61-aa regulatory peptide at the N-terminus. Prior to expression of the mature gp63 protein on the cell surface, the precursor gp63 protein is processed in *Leishmania* to remove the pre-pro regions from the N-terminus and a hydrophobic peptide from the C-terminus is replaced by a glycosyl-inositol phospholipid tail . To produce the mature form of recombinant gp63 in *E. coli*, the cloned *L. major* gp63 gene was modified using the Polymerase Chain Reaction (PCR) technique to eliminate the coding regions for the peptides that are removed during processing of the gp63 precursor protein in *Leishmania*. The PCR modified gp63 gene from *L. major* was expressed at high-levels as a nonfusion protein in an *E. coli* expression system (>30% total protein) in which the cloned gene is regulated by a promoter from phage T7 and is selectively expressed by T7 RNA Polymerase in the *E.coli* host. Gp63 genes from diverse species of *Leishmania* are highly conserved as shown by hybridization studies with the *L. major* gene. Using PCR, total genomic DNA, and the oligodeoxyribonucleotide primers that were designed for the *L. major* gp63 gene, the gp63 genes from both *L. donovani* and *L.b. braziliensis* were amplified and modified to delete the coding regions for the precursor peptides. High levels of recombinant gp63 were produced in *E. coli* for both the *L. donovani* and *L.b. braziliensis* genes using the T7 RNA Polymerase expression system.

O 409 THE UBIQUITIN GENES OF TRYPANOSOMA BRUCEI.
David A. Campbell and Sandy Wong, Microbiology and Immunology, University of California at Los Angeles, CA90024.

The genome of Trypanosoma brucei contains at least four discrete regions which hybridise with a cloned ubiquitin repeat. Two of the regions encode polyubiquitin proteins, containing approximately 31 and 12 head-to-tail, spacerless ubiquitin repeats (228bp) respectively. Polyubiquitin transcripts are detectable at a higher level in bloodstream forms than procyclics. The level of the largest transcript may be increased by heat-shock. A third region of hybridisation contains two identical, tandem genes, which encode a protein predicted to contain a single ubiquitin repeat fused to a carboxy extension with a zinc-finger motif.

O 410 TRANSLATIONAL INITIATION AT *UAG* IN A FUNCTIONAL GENE OF PLASMODIUM FALCIPARUM, Ulrich Certa and Paola Ghersa*, Central Research Units, F. Hoffmann-La Roche & Co. Ltd., CH-4002 Basle and *GLAXO I.M.B., 1227 Geneva, Switzerland.

Initiation of translation in pro- and eukaryotes conventionally requires a AUG codon. In procaryotes a tRNA molecule loaded with formyl-methionine recognizes the signal and the transcript is translated. The gene encoding fructose-1,6-diphosphate aldolase of Plasmodium falciparum has been cloned by us and expression of the protein in bacteria or yeast consistently resulted in the synthesis of a slightly smaller protein compared to the parasite's enzyme. No additional AUG in frame is found upstream of this startcodon and we show that translation initiates at a UAG amber codon. The evidence includes sequences from 4 independent cDNA and genomic clones containing the relevant region. Direct mRNA sequencing rules out the existence of an intron containing the putative AUG. In vitro translation experiments using poly-T selected mRNA show that a rabbit reticulocyte lysate faithfully recognizes the UAG-initiation signal. Other experiments rule out the existence of a *UAG-f-MET*-suppressor tRNA and the secondary structure at the 5'-end of the aldolase transcript may be involved in this novel initiation of translation.

O 411 IMMUNOSELECTION OF cDNA CLONES ENCODING DIAGNOSTIC ANTIGENS: A Trypanosoma cruzi SPECIFIC ANTIGEN CONTAINS A 7 AMINO ACID TANDEM REPEAT, J. Louise Clarke and Michael A. Miles, Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.

A dual ELISA procedure was developed for the differential primary immunoselection of species-specific clones from cDNA expression libraries in λgt11. This method was used with sera from patients with visceral leishmaniasis or Chagas' disease to isolate a series of clones encoding putatively diagnostic antigens. One of the clones [5A-1B] contained a 374 bp insert and encoded a fusion protein recognised by sera from Trypanosoma cruzi infected patients but not by sera from Leishmania infected patients. DNA sequence analysis revealed a 7-amino acid tandem repeat adjacent to the -COOH terminus of the protein. Synthetic peptides and epitope mapping are being used to define the antigenic determinants.

O 412 CLONING AND EXPRESSION OF SURFACE-ASSOCIATED ANTIGENS OF ONCHOCERCA volvulus MICROFILARIAE, Franz J. Conraths, William Harnett and R. Michael E. Parkhouse*, Divisions of Parasitology and Immunology*, National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA, United Kingdom.
Surface antigens of Onchocerca microfilariae are potential targets for both protective and immunopathological immune responses. To investigate the properties of antigens of the microfilarial epicuticle, four monoclonal antibodies to O. gibsoni and O. lienalis microfilariae have been prepared and characterized by immunochemical procedures (immunofluorescence, immunoprecipitations, Western blotting). They react with the surface of uterine microfilariae, but fail to recognize skin microfilariae in immunofluorescent assays. However, radio-iodinated antigen derived from surface-labelled skin microfilariae can be immunoprecipitated. These findings may indicate that the epitopes recognized become masked after release of the microfilariae from the uterus. In Western blot experiments, the antibodies react with antigens of 14 kD (two antibodies), 29/31 kD, and 44/60 kD. They were successfully employed to screen a lambda-gt11 cDNA expression library of adult female O. volvulus (provided by Dr. J. Donelson, Iowa, U.S.A.) and the resulting beta-galactosidase fusion proteins were used to determine human antibody responses to these antigens. Experiments in progress are directed towards DNA sequencing, and testing the expressed proteins for immunisation and protection of infected mice.

O 413 GENE EXPRESSION IN THE INFECTIVE PROMASTIGOTES OF *LEISHMANIA MAJOR*.
Richard M.R. Coulson, Susan Searle, Helen Flinn and Deborah F. Smith, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ, U.K.
We are studying the molecular events which accompany the differentiation of *Leishmania major* promastigotes, in order to facilitate the development of prophylactics against infective-stage organisms, which are the causative agents of human cutaneous leishmaniasis. By differentially screening cDNA libraries, we have identified a number of nuclear genes whose expression is up-regulated in infective-stage promastigotes, including a putative ATPase, a specific β-tubulin and a small family of genes with homology to heat shock protein 70. These hsp-like genes, which encode proteins of 70-75 Kd, are dispersed on different chromosomes in *Leishmania* and show either developmental or constituitive expression. Two other genes show differential expression patterns during the parasite life cycle: cDNA 2 and cDNA 14 are up-regulated in infective promastigotes but, while cDNA 2 shows greatly elevated expression in amastigotes, the cDNA 14 mRNA is barely detectable. We are currently using fusion proteins and their antibodies to identify the native proteins encoded by these genes and their location within the cell, prior to biochemical studies. In addition, we are analysing the genomic organisation of these sequences (some of which are repeated), as a preliminary to the study of the relative importance of transcriptional signals and post-transcriptional processing in the regulation of gene expression in these organisms.

O 414 CHARACTERIZATION AND PARTIAL PURIFICATION OF THE AVERMECTIN BINDING-SITE FROM CAENORHABDITIS ELEGANS. Doris F. Cully, Philip S. Paress, James M. Schaeffer and Mervyn J. Turner, Merck & Co., Rahway, NJ 07065.
Avermectins (AVM) are a class of macrocyclic lactones isolated from Streptomyces avermitilis, which have strong anthelmintic activity. We have used the free living nematode Caenorhabditis elegans, which is sensitive to the avermectins, as a model of parasitic nematodes. Specific binding sites for IVM have been identified in C.elegans membrane fractions. We have extracted a high affinity IVM binding-site with n-octyl β glucoside (NOG). Scatchard analysis indicates that the NOG-solubilized material has a K_D for IVM of 0.48 nM and a B_{max} of 0.14 pmole/mg protein, while the starting membrane fraction has a K_D of 0.55 nM and a B_{max} of 0.24 pmole/mg protein. Kinetic analysis of IVM binding shows that both the solubilized and membrane bound binding-sites form a slowly reversible complex with IVM. Purification of the IVM binding-site has been attempted by anion exchange, size exclusion, chromatofocusing and affinity chromatography. The identification of a peptide associated with IVM binding activity will enable the synthesis of a probe to be used for the cloning of the binding-site gene.

O 415 SCHISTOSOMA MANSONI HEMOGLOBINASE IS PROCESSED FROM A 50 KD FORM TO THE MATURE 31 KD ENZYME.

Richard E. Davis, Nelson B. Phillips, M.A. EL-Meanawy and Alan H. Davis, Department of Molecular Biology and Microbiology and the Department of Medicine, Case Western Reserve University, Cleveland, Ohio 44106. *Schistosoma mansoni* worms consume large numbers of host red blood cells. The hemoglobin contained therein is thought to be an essential worm nutrient. Hemoglobin degradation is catalyzed by an acidic, cysteine, 31 kd *S. mansoni* protease, known as hemoglobinase. Information on the structure and pharmacology of this protease could lead to the development of new chemotherapeutic agents effective against schistosomes. We determined the amino acid sequence of the protease from the complete hemoglobinase mRNA sequence derived from cDNA sequences and primer extension sequencing of the hemoglobinase mRNA. Translation of the complete hemoglobinase mRNA sequence predicted proteins larger in size, 50 kd, than the purified 31 kd hemoglobinase. These data suggested that hemoglobinase could be synthesized as a proenzyme. Hybrid arrested, *in vitro* translation and immunoprecipitation experiments confirmed that the primary translation product of hemoglobinase mRNA was 50 kd. Furthermore, proteins of 50 kd were observed in worm lysates in Western blots using anti-hemoglobinase sera. The hemoglobinase proenzyme contains amino-terminal residues characteristic of a signal sequence followed by amino acids identical to the amino-terminus of the purified, 31 kd enzyme. Simple removal of the signal could not account for the size differences between the proenzyme and the mature enzyme. Sequencing of the carboxy-terminus of the purified 31 kd protease with carboxypeptidase Y revealed that 138 terminal amino acids are removed from the carboxy-terminus of the proenzyme to produce the mature 31 kd protease. Computer-assisted comparisons of the sequence of the 31 kd hemoglobinase with known proteases did not reveal any homologies. Expression of the proenzyme in bacteria indicated that the recombinant enzyme was active at low levels. Thus, construction of clones coding for the mature 31 kd enzyme may allow the screening and development of novel chemotherapeutic anti-schistosomal agents.

O 416 PRIMARY STRUCTURE OF THE MAJOR MEROZOITE SURFACE ANTIGEN OF P. CHABAUD CHABAUDI,

W. Deleersnijder, D. Hendrix, N. Bendahman, J. Hanegreefs, C. Hamers-Casterman and R. Hamers, Vrij Universiteit Brussel, Instituut voor Moleculaire Biologie, Paardenstraat 65, 1640 Sint-Genesius-Rode, Belgium

The gene for the major merozoite surface antigen (MSA) of the rodent malaria parasite Plasmodium chabaud chabaudi has been cloned and sequenced. A single open reading frame was found coding for 1609 amino acid (AA). The translated sequence starts with a 19 AA long signal peptide and ends with a hydrophobic anchor sequence. Tandem oligopeptide repeats were found at 4 different positions in the protein. The largest repeat was found near the aminoterminal end. It comprises an array of 11*8 AA. Further downstream, repeats of 2*9AA, 4*4AA and 2*4AA are found. The amino acid sequence of the P.c.c. MSA shows 75% homology with the known 700 AA at the carboxyterminal end of the P. yoelii MSA and 32% homology with the MSA of P. falciparum (p195). The predicted molecular weight of the translated sequence is 178,000. This contrasts with an anticipated Mr of 230,000 as estimated by SDS-PAGE. By scoring the reactivity of a set of 15 anti6MSA monoclonal antibodies against a number of overlapping cDNA expression clones, a provisional epitope map of the protein was established. The binding sites of all monoclonals were clustered in the middle third of the protein.

O 417 GLYCOSYL PHOSPHATIDYL INOSITOL ANCHOR OF TOXOPLASMA GONDII MAJOR SURFACE PROTEINS,

Jean F. Dubremetz, Stanislas Tomavo, Ralph T. Schwarz, U42 INSERM, 369 Rue Jules Guesde, 59650 Villeneuve d'Ascq, France.

The four major surface antigens (MSAs: P43, P35, P30, P22) of Toxoplasma gondii tachyzoites can be removed from living organisms and converted into soluble form by phosphatidyl inositol specific phospholipase C (PIPLC) treatment. Quantitative removal of lactoperoxidase radioiodinated MSAs can be obtained and solubilized antigens can be individually immunoprecipitated by specific monoclonal antibodies. A sligh electrophoretic shift can be observed between native and PIPLC treated MSAs when analyzed by SDS-PAGE. T. gondii MSAs can be biosynthetically labeled with radioactive amino acids, glucosamine, mannose, palmitic acid and myristic acid. Ethanolamine labeling has been obtained so far for P30 and P22. Fatty acid labeling of MSAs can be removed by PIPLC treatment. PIPLC solubilized T. gondii MSAs are recognized on western blots by anti CRD antibodies (raised against Trypanosoma brucei soluble VSG) whereas native MSAs are not. Polyclonal antibodies raised against affinity purified P43 cross react with the soluble forms of P35, P30 and P22 but not with native antigens. These results strongly suggest that P43, P35, P30 and P22 are anchored in T. gondii tachyzoite membrane by a glycosyl phosphatidyl inositol structure.

O 418 APPROACHES TO AN IN VITRO ASSAY FOR PROTEIN TRANSPORT IN ERYTHROCYTIC STAGES OF *PLASMODIUM FALCIPARUM*. Heidi G. Elmendorf and Kasturi Haldar. Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford CA 94305.

The intraerythrocytic malaria parasite transports proteins to the erythrocyte membrane which appear to modify properties of the host cell membrane. To study the regulation of molecular trafficking between host and parasite membranes we are reconstituting transport of plasmodial proteins from "free parasites" to exogenously added erythrocyte vesicles, *in vitro*. Using a stainless steel-ball homogeniser intraerythrocytic trophozoites of *P. falciparum* FCR-3/A_2 can be selectively freed from their host cell membranes. Ring infected and uninfected erythrocyte membranes are not ruptured. Over 90% of the original trophozoites are released as intact free parasites and are seperated from other components by density centrifugation over percoll. Over 99% free parasites, (with the remaining contamination from ghosts) are obtained. The purified free parasites are stable for several hours in their homogenisation buffer and additional working buffers designed to mimic the intracellular environment, but not in RPMI 1640. The extra-erythrocytic trophozoites are capable of active synthesis of plasmodial proteins. Studies are in progress to follow transport of these radiolabelled proteins to exogenously added erythrocyte membranes and the effect of cytosolic components on protein transport.

O 419 INTRASPECIFIC DIFFERENCES IN THE OXIDATIVE METABOLISM OF Trypanosoma cruzi. J.C. Engel, P.S. Doyle and J.A. Dvorak. Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD. 20892. T. cruzi, a parasitic protozoan, metabolizes carbohydrates to CO_2 and partially reduced catabolites (i.e. succinate, acetate). A branched electron transport chain involving cytochrome (cyto.) aa_3 and cyto.o as terminal oxidases has been described. The organism displays high intraspecific heterogeneity. This study was performed to determine if heterogeneity extends to the level of oxidative metabolism in the epimastigote stage of two cloned stocks, CA-I/72 and HO-3/15. A comparative study of cyto. contents, respiratory rates and sensitivity to salicylhydroxamic acid (SHAM) showed that: clone CA-I/72 contains 4 times more cyto.b than HO-3/15. Cyto.o was not detected in HO-3/15 cells but was present at a concentration similar to cyto.b in CA-I/72. The stimulation of oxygen uptake by glucose following glucose starvation was higher in HO-3/15 than CA-I/72. The addition of glucose in the presence of Antimycin A (Anti-A) to glucose-starved cells resulted in an increase in oxygen utilization in CA-I/72; glucose-starved HO-3/15 cells were not influenced by the presence of Anti-A. SHAM produced an additional 50% reduction in oxygen uptake in cells partially inhibited with Anti-A independent of the growth phase of the cells. These data indicate that marked intraspecific metabolic differences also exist within T. cruzi and that an α-glycerophosphate oxidase or similar SHAM-inhibitable compound is present in the organism.

O 420 SECONDARY STRUCTURE OF THE PROMASTIGOTE SURFACE PROTEASE OF *LEISHMANIA*. Robert Etges and Fritz Jahnig, Max-Planck-Institut für Biologie, Corrensstr. 38, D-7400 Tubingen 1, Federal Republic of Germany. By Raman spectroscopic analysis we have determined the secondary structure of the Promastigote Surface Protease, or PSP, of *Leishmania major* LEM-513. It consist of nearly 50% antiparallel ß-strand, and less than 20% α-helix. These results are contrasted with the predominantly α-helical VSGs of the African trypanosomes and the α-helical metalloprotease thermolysin. The PSP of *Leishmania* thus represents a novel class of membrane-anchored metalloprotease.

O 421 POLYMORPHISM OF PLASMODIUM FALCIPARUM PROTEINS AND GLYCOPROTEINS, Brian Fenton, Alison Creasey, John Clark, Alison Walker, Jana McBride, Richard Carter and David Walliker. Departments of Genetics and Zoology, The University of Edinburgh, West Mains Road, Edinburgh, EH9 3JN, Scotland.

Proteins of Plasmodium falciparum asexual and gametocyte stages have been examined by (i) metabolic labelling of cultured parasites followed by two-dimensional (2D) electrophoresis, and (ii) immunofluorescence and immunoblotting with monoclonal antibodies. Glycoproteins have been identified by the use of radio-labelled glucosamine. A glycoprotein associated with the merozoite surface, denoted MSA2, is polymorphic in different parasite isolates. Seven allelic forms of this glycoprotein has been detected, varying in size from 35kDa to 48kDa, in isoelectric point from 4.6 to 5.2, and in immunological specificity. One form of MSA2, exhibited micro-heterogeneity on 2D gels, more than spot being seen in a given parasite clone. Such micro-heterogeneity is also seen in a gametocyte specific antigen denoted Pfsi 25-27. The causes of this variation are not known, but could include glycosylation, phosphorylation or myristilation of the protein.

O 422 SURFACE-EXPOSED MEMBRANE PROTEINS OF TOXOPLASMA GONDII AND THEIR INTERACTION WITH LAMININ, Stephen A. Fuhrman, Glaucia C. Furtado, Hynda Kleinman and Keith A. Joiner, Laboratory of Parasitic Diseases, NIAID and Laboratory of Developmental Biology and Anomalies, NIDR, NIH, Bethesda, MD 20892

We have examined the surface exposed membrane proteins of Toxoplasma gondii, an obligate intracellular protozoan which has gained in medical importance since the advent of AIDS. The membrane of the tachyzoite stage has been considered relatively simple, consisting of four major surface iodinatable constituents (p41-43, p35, p30, and p22) which are solubilized by non-denaturing detergents. We developed a rapid separation scheme for these four constituents using two-step chromato-focusing on a fast protein liquid chromatography system. Three of these constituents (p41-43, p30, and p22) are integral membrane proteins, as determined by Triton X-114 phase separation. Several heretofore unrecognized surface-iodinated or -biotinylated proteins of the tachyzoite membrane, however, are not solubilized by non-denaturing detergents. Of these, the most prominent constituents of 67, 60, 16, and 14 kDa bind the extracellular matrix protein laminin, which we have recently found enhances T. gondii attachment and entry into cells. The enhancement of infectivity is blocked by the peptide YIGSR, which is directed to a cell attachment site of laminin. Immunoblot analysis shows the 67/60 kDa doublet to be immunologically related to the mammalian non-integrin 67 kDa laminin binding protein[1-3], and may be derived from this source. These data show that several previously unrecognized membrane constituents of tachyzoites are surface exposed, bind laminin, and may be host cell derived and important in cell attachment and invasion.

O 423 CHARACTERIZATION OF A PLASMODIUM VIVAX PROTEIN WHICH IS LOCALIZED AT THE APICAL END OF THE MEROZOITE, Mary R. Galinski and John W. Barnwell, Department of Medical and Molecular Parasitology, New York University Medical Center, New York, N.Y. 10010

Invasion of erythrocytes by plasmodia begins after merozoites orient themselves such that their apical ends are juxtaposed with the red blood cell membrane. We have identified a protein of 250 kd from P. vivax blood stage parasites that may be involved in this interaction. A portion of the gene encoding this protein (clone P.v. A) was identified via screening a lambda gt11/P.vivax genomic DNA expression library with serum from a Saimiri monkey hyperimmune to P. vivax blood stage parasites. Antibodies from this serum, which reacted specifically with clone P.v. A were purified and used for immunofluorescence analysis (IFA) and to precipitate parasite proteins from 35S Methionine labeled parasite extracts. A protein of 250 kd was immunoprecipitated and IFA generated a unique fluorescing pattern at the apical end of the merozoite. Sequence data obtained to date (4kb) suggests that this is a very highly charged protein. Studies are now in progress to complete the sequence of the entire gene that encodes this protein, to assess the functional role of this protein in invasion, and to obtain its precise localization by immunoelectron microscopy.

O 424 LIPID TRANSPORT FROM THE ERYTHROCYTE MEMBRANE TO THE PARASITE IN *PLASMODIUM FALCIPARUM* INFECTED CELLS. Kasturi Haldar*, Angela F. de Amorim* and George A. M. Cross+. *Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford CA 94305 and +Department of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, N. Y. 10021.

The asexual development of the human malaria parasite *Plasmodium falciparum* is largely intraerythrocytic. When 1-acyl-2-(-4-nitrobenzo-2-oxa-1,3-diazole)-aminocaproyl phosphatidylcholine (NBD-PC) was incorporated into infected and uninfected erythrocyte membranes at 0°C, it remained at the cell surface. At 10°C, the lipid was rapidly internalised in infected erythrocytes at all stages of parasite growth. Our results indicate that the internalisation of NBD-PC was not due to endocytosis, but to rapid transbilayer lipid flip flop at the infected erythrocyte membrane, followed by monomer diffusion to the parasite. Internalisation of the lipid was inhibited by i) depleting cellular ATP levels, ii) pretreating the cells with N-ethyl maleimide or diethylpyrocarbonate, and iii) 10 mM L-α-glycerophosphorylcholine, suggesting protein and energy dependent transmembrane movement of the analogue. The conditions for the internalisation of another phospholipid analogue N-4-nitrobenzo-2-oxa-1,3-diazoledipalmitoyl phosphatidylethanolamine (N-NBD-PE) were distinct from that of NBD-PC, and suggest the presence of additional mechanism/s of parasite mediated lipid transport in the infected host membrane. Inspite of the lack of bulk, constitutive endocytosis at the red cell membrane, the uptake of lucifer yellow by mature infected cells suggests that microdomains of pinocytotic activity are induced by the intracellular parasite. The results indicate the presence of parasite induced mechanisms of lipid transport in infected erythrocyte membranes, which modify host membrane properties and may have important implications on phospholipid asymmetry in these membranes.

O 425 CLONING AND CHARACTERISATION OF SURFACE ANTIGENS OF SCHISTOSOMA MANSONI, Jane C. Havercroft, Maureen C. Huggins, Angela L. Smith and David W. Taylor, Department of Pathology, University of Cambridge, Cambridge CB2 1QP, United Kingdom.

Surface antigens of the Schistosomula of S. mansoni appear to be a target for the immune attack. Consequently, we are attempting to clone such potentially protective antigens with a view to testing their use in an experimental vaccine. A cDNA library in λgtII was screened with a pool of sera known to recognise schistosomulum surface epitopes. Among the positive clones obtained, two are of particular interest: one represents a 20Kd polypeptide which has homology to the calmodulin family of calcium binding proteins. The second encodes a 50Kd antigen. An antiserum raised against the fusion protein of this clone gives positive indirect immunofluorescence of the surface of schistosomula in indirect immunofluorescence assays on intact organisms. In addition, it is able to mediate killing of schistosomula by eosinophils in vitro. This suggests that this clone encodes a surface antigen and the possibility that it can protect against infection in vivo will be investigated.

O 426 A cAMP INDUCIBLE GENE EXPRESSED DURING THE DEVELOPMENT OF INFECTIVE STAGES OF TRYPANOSOMA CRUZI, Stephen Heath, Kenneth Vernick, Sara Hieny and Alan Sher, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892. Trypanosoma cruzi undergoes an important developmental change (metacyclogenesis) immediately before leaving the insect vector. This transformation pre-adapts the parasite for survival in the vertebrate host. The differentiation of infective metacyclic trypomastigotes from non-infective epimastigotes can be duplicated in vitro by allowing epimastigotes to grow to stationary phase. This process has been previously been shown to be influenced by cAMP. We have employed subtractive hybridization techniques to identify several cDNA clones which are expressed preferentially in metacyclic stages. One of these, TC43.1 (derived from T. cruzi strain Sylvio X10/4) has been characterized in detail. Transcription of TC43.1 can be induced in epimastigotes by cyclic AMP (cAMP) and the cAMP analogues dibutyryl cAMP and 8-bromo-cAMP, but is inhibited by imidazole, an activator of cAMP dependent phosphodiesterase. The deduced amino acid sequence of the cDNA clone demonstrates a 42% homology in a 35 amino acid overlap with the proto-oncogene c-fos which is itself a cAMP inducible gene. While present in the genome as a single copy, Northern analysis indicates that TC43.1 produces multiple transcripts of 3.2, 2.2 and 1.8 kb suggesting the occurrence of alternate splicing. The data taken together suggest that TC43.1 encodes a cAMP dependent regulatory protein involved in the differentiation of T. cruzi infective forms.

O 427 IDENTIFICATION OF AN EGF RECEPTOR HOMOLOGUE IN TRYPANOSOMES, Geoff Hide, Alex Gray and Andrew Tait, Wellcome Unit of Molecular Parasitology, Department of Veterinary Parasitology, Bearsden Road, Glasgow, G61 1QH, Scotland, United Kingdom.

Our understanding of growth regulation in trypanosomes has yet to be elucidated at the molecular level. One possible mechanism involves the interaction of these parasites with host or vector growth factors. In this work, we report evidence for a growth factor receptor homologue in trypanosomes, thus implying that such a mechanism may be involved in trypanosome growth regulation. Antibodies, which recognise the mammalian EGF receptor, cross-reacted, by Immunofluorescence and immune precipitation, with Trypanosoma brucei. A surface polypeptide of 135kd was identified by the antibody and was found to be present in both the procyclic and bloodstream stages. Protein kinase activity was found to be associated with the immune precipitate. Crosslinking experiments have demonstrated that EGF binds specifically to trypanosomes and that the 135kd polypeptide as the surface molecule that binds the EGF. Another polypeptide of 50kd was also found to bind EGF. EGF was found to modify protein kinase activity in membrane fractions and several proteins, including the 135kd polypeptide, were phosphorylated to a greater extent in the presence of EGF. Addition of EGF also increased growth rate of trypanosomes in vitro. Thus, it can be concluded that a surface polypeptide with considerable homology to the mammaliam EGF receptor exists in trypanosomes. This finding has implications for a novel mechanism of host parasite interaction.

O 428 PARTIAL PURIFICATION OF THE α GLYCEROPHOSPHATE OXIDASE FROM TRYPANOSOMA BRUCEI, **George C. Hill and Harry Bass, Division of Biomedical Sciences, Meharry Medical College, Nashville, TN 37208.** **Bloodstream African trypanosomes are completely dependent on glycolysis for their energy supply and utilize a terminal oxidase, α glycerophosphate oxidase, to reoxidize the glycolytically produced NADH. This terminal oxidase is cytochrome independent, not inhibited by classical inhibitors of the respiratory chain, inhibited by salicylhydroxamic acid and consists of two components: a flavin-linked glycerol-3-phosphate dehydrogenase and a glycerol-3-phosphate oxidase (GPO) which are probably linked by ubiquinol. Partial purification of Trypanosoma brucei GPO has been accomplished. Mitochondria from bloodstream trypomastigotes were treated with 7.5mM lauryl maltoside to release the GPO from the mitochondrial membrane. Solubilized protein was applied to a Pharmacia Mono Q HR 5/5 anion exchange column and chromatography was performed at 4°C with a FPLC system. All fractions were measured for enzyme activity with 10mM glycerol-3-phosphate or 0.6 mM of an ubiquinol analog as substrate. Ubiquinol oxidase was eluted from the column with 300-400mM KCl and a 30 fold enrichment for this oxidase was obtained. Enzyme activity was inhibited 100% by 0.5mM SHAM, thus confirming the oxidase activity. 30% enzyme activity and 85% of the protein were recovered from the column.** **Supported by Army Contract DAMD 17-86-C-6077 and NIH Grant AI 21159.**

O 429 CHARACTERIZATION OF HYDROGENOSOMES AND GENES WHICH ENCODE HYDROGENOSOMAL PROTEINS OF TRICHOMONAS VAGINALIS, Patricia J. Johnson[1] and Miklòs Müller[2] Department of Microbiology & Immunology, UCLA School of Medicine, Los Angeles, CA 90024[1], Department of Biochemical Parasitology, Rockefeller University, New York, NY 10021[2]

The hydrogenosome, an unusual organelle found in Trichomonas vaginalis, plays a central role in the metabolism and drug susceptibility of this human-infective parasite. To gain a better understanding of the origin and biogenesis of this organelle, stable hydrogenosomes have been highly purified and used to immunize rabbits. The resulting antisera were used to screen a cDNA expression library in lambda gt11. Additionally, the library was screened with antibodies against purified hydrogenosomal proteins. We present these data and a molecular analysis of these genes - the first to be cloned and characterized from a trichomonad.

O 430 TRYPANOSOMA CRUZI MAMMALIAN STAGE SPECIFIC TRANSCRIPTS REVEAL AREAS OF SEQUENCE HOMOLOGY AND DIVERSITY, Stuart J. Kahn, Wesley Van Voorhis, and Harvey A.Eisen, Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98104. Trypanosoma Cruzi randomly sheared DNA, was cloned into a lambda GT-11 expression library,and screened with sera from T. Cruzi chronically-infected mice. A selected clone by Southern analysis revealed homology with greater than 25 fragments of T. Cruzi genomic DNA cleaved with 10 different restriction enzymes. Northern analysis revealed a single band at 4.5 kilobases. The band was detected in the Trypomastigote poly(A)+ RNA, but not the Epimastigote. Several cDNA clones were selected by hybridization to a fragment of this gene. Analysis of these cDNA clones revealed a family of mRNA, all with identical size of 4.5 kilobases, and significant areas of sequence homology and divergence within the open reading frame.

O 431 **REARRANGEMENT SITE FOR H REGION AMPLIFICATION CONFERRING MULTIPLE DRUG RESISTANCE IN LEISHMANIA IS A DISPERSED REPETITIVE ELEMENT** Paul F. Kamitsuka, David Iovannisci, Thomas E. Ellenberger, and Stephen M. Beverley, Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115. L. major may become resistant to chemotherapeutic agents through gene amplification. H region amplification is of particular interest as it confers multiple drug resistance (not by decreased drug accumulation) . We have been studying the structural characteristics of this region in an effort to shed light on the mechanisms of its amplification. The H region is flanked on both sides by pairs of inverted homologous DNA segments, which themselves flank a central transcribed region (Fig. A). We have shown by hybridization analysis that the inverted segments at the left end of the H region represent dispersed repetitive elements, found on most chromosomes resolvable by PFGE. These properties suggest a transposable element, although the central transcribed region is not dispersed. Amplification involves homologous recombination between the inverted repeat regions of two wild-type chromosomes arranged in opposite orientation, yielding a circular amplified species containing two copies of the H region (Fig. B).

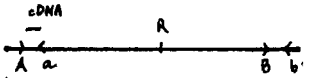

Fig. A.

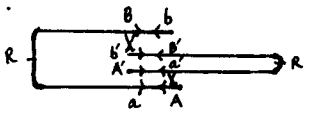

Fig. B.

O 432 MYC-HOMOLOGUES IN TRYPANOSOMA BRUCEI, Choong H. Kim, M.E.M. Colmenauer, Donald G. Guiney and Charles E. Davis, Departments of Pathology, Medicine, and Center for Molecular Genetics, University of California at San Diego, CA 92103
We have shown previously that: 1) dibutyryl cAMP and other agents that elevate T. brucei cAMP induce differentiation from the slender to the stumpy bloodstream form, and 2) difluoromethylornithine, an inhibitor of polyamine synthesis, induces differentiation without elevating cAMP. Since these agents induce differentiation of higher eukaryotes by decreasing c-myc expression, we assayed T. brucei for myc family homologues. Immunoblots of slender, stumpy, and procyclic (insect) forms revealed two proteins that react with myc family antibodies. Southerns of EcoRI-digested procyclic DNA probed with v-myc contained 3 homologous bands, including an 8 kb sequence that also hybridized to a 25-mer oligonucleotide synthesized according to a consensus sequence in the second exon of the myc family. Further restriction analysis revealed that the homologous region was contained within a 1.0 kb BamH1 fragment which we cloned to use as a probe. The homologous 8 kb EcoRI fragment was present in all 3 life cycle stages, suggesting that the gene is not rearranged during differentiation. Northerns of procyclics cultured in the presence of fetal calf serum showed that the myc homologue is expressed as a 3.2 kb transcript. We are currently sequencing the DNA homologue and comparing expression of the mRNA transcript in all 3 life cycle stages to confirm the presence, and assess the possible significance, of a myc-family consensus sequence in T. brucei.

O 433 MOLECULAR STUDIES OF TWO SCHISTOSOMA MANSONI PROTEASES: CATHEPSIN B AND "HAEMOGLOBINASE", Mo-Quen Klinkert, Richard Felleisen, Kurt Bommert, & Ewald Beck, ZMBH, University of Heidelberg, FRG.

Two highly immunogenic S. mansoni adult worm proteins Sm31 and Sm32 have been identified as Cathepsin B and a haemoglobinase, respectively. One of our interests in these molecules is to investigate their possible use in diagnosis of schistosomiasis. Both antigens have been cloned and expressed in a plasmid expression vector pEx34 in E.coli as hybrid proteins fused to MS2 RNA polymerase. The fusion proteins have been tested in TSP-ELISA on a large number of human infection sera and found to be very promising diagnostic reagents. In addition antigenic determinants on the proteins were localised by a deletion mutation procedure and the corresponding antigenic regions were synthesised as oligo peptides. Preliminary results suggest that some of these peptides may represent important immunodiagnostic probes. Well-defined peptides can be easily synthesised, and their use may greatly eliminate background problems often encountered in serological assays. To ensure the expression of all putative antigenic sites, the complete sequences were cloned in an eucaryotic viral vector. Screening of recombinant viruses expressing the "authentic" forms of the two proteases is in progress. The full-length sequences of both antigens were cloned in pSP65. Data from *in vitro* translation studies of the cathepsin B construct suggest that the precursor preprocathepsin B is processed *in vitro*. From the "haemoglobinase" RNA a precursor form which was not further processed was observed. In contrast to cathepsin B, *in vitro* glycosylation of "haemoglobinase" could be demonstrated in the presence of canine pancreatic membranes.

O 434 A 60 kDA PLASMODIUM FALCIPARUM PROTEIN AT THE MOVING JUNCTION FORMED BETWEEN MEROZOITE AND ERYTHROCYTE DURING INVASION, Francis W. Klotz and Louis H. Miller, Department of Immunology, Walter Reed Army Institute of Research, Washington DC, 20307-5100 and Laboratory of Parasitic Diseases, National Institute of Allergy and Infections Diseases, National Institute of Health Bethesda, Md. 20892.

Invasion of erythrocytes by malaria merozoites requires the formation of a junction of attachment between erythrocyte and merozoite membranes. The attachment junction initially forms at the apical region of the merozoite. It then moves around to the posterior of the merozoite as invasion proceeds. A monoclonal antibody against a 60 kDa merozoite protein (termed MCP-1 for Merozoite Capping Protein 1) of Plasmodium falciparum reacts in an immunofluorescence pattern resembling the moving junction. By two-color immunofluorescence, MCP-1 was located at the attachment site formed between the merozoite apical region and erythrocyte. During invasion, MCP-1 separated and migrated around merozoites at the orifice of the parasitophorous vacuole. In newly-invaded erythrocytes, MCP-1 persisted at the pole of the young parasite nearest the erythrocyte membrane, suggesting its anterior-to-posterior movement. MCP-1 exhibited no variability in molecular mass among the FCR-3, Camp, and 7G8 strains of P. falciparum, and the epitope was invariant in the P. falciparum strains studied. We conclude that MCP-1 may participate in merozoite invasion of erythrocytes by facilitating attachment or movement of the junction along the parasite cytoskeletal network.

O 435 PRESENCE OF REPEATED SEQUENCES IN EIMERIA TENELLA, Christine Ko[1], Joe Cox[1], Ken Bafundo[2], Chuck Smith[2], Mary Stiff[2], Michael McDonell. Synergen, Inc., Boulder, CO[1]; Greenfield Lab., Eli Lilly & Co., Greenfield, IN[2]. Poultry coccidiosis, caused by six major Eimeria species, is presently controlled almost exclusively by prophylactic chemotherapy. However, because of increasing signs of evolving drug resistance and concern over possibility of drug residues in poultry products, alternative methods of control is desirable. During the attempt to develop a genetically-engineered vaccine against coccidiosis, we isolated serum-reactive phages from Eimeria tenella genomic and cDNA expression libraries using immune chicken sera and hyperimmune rabbit sera prepared against E. tenella sporozoites. We report here that a surprisingly large number of the recombinant phages harbor DNA sequences encoding either polyamino acids or tandemly repeated amino acids. We show that the DNA repeats may be translated in different reading frames. These sequences are highly repeated in the genome of E. tenella and of several other Eimeria species. However, the sequences are Eimeria-specific, and are not repeated in Caenorhabditis elegans, Drosophila melanogaster, Saccharomyces cerevisiae, human or chicken genome. Both polyamino acid or tandemly-repeated amino acids sequences have been widely reported in the malarial parasite Plasmodium, which like the coccidia, are both protozoa of the phylum Apicomplexa. It has been reported that the malarial repeats are targets of the host's immune responses and are also prime candidates for malarial vaccines. The Eimeria repeated sequences reported here may play a similar role in immunity.

O 436 IMMUNOCHEMICAL STUDIES ON A *PLASMODIUM FALCIPARUM* 102 KD PROTEIN: THE PUTATIVE TRANSFERRIN RECEPTOR OF INFECTED ERYTHROCYTE MEMBRANE. Wen-lu Li and Kasturi Haldar, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305

Plasmodium falciparum infected erythrocytes require noneheme iron for growth. Previous studies showed that parasitized erythrocytes contain internalized transferrin (Rodrigues-Lopez,M.H. et al, 1986, Nature 324,388-391) and a parasite protein of 102 kd binds human transferrin in the soluble complex (Haldar, K. et al, 1986, Proc. Natl. Acad. Sci. USA 83, 8565-8569). Parasite lysis prepared in 0.1% trifluoroacetic acid containing 102 kd transferrin binding protein was used to immunize BALB/c mice. On fusion of the spleen cells from immunized mice with Ag8-653 myeloma cells, a hybridoma secreting monoclonal antibody to 102 kd protein has been obtained. A pattern of small dots observed on immunofluorescence assay suggests the vesicular localization of this protein. Studies about the function of the immunopurified 102 kd protein are in progress.

O 437 A CYSTEINE PEPTIDASE CONSERVED AMONGST TRYPANOSOMATIDS, Catherine M. Lowndes and Frank Ashall, London School of Hygeine and Tropical Medicine, Keppel Street, London WC1E 7HT, U.K.

A range of trypanosomatids were examined for a cysteine peptidase activity previously detected in T.cruzi. Detergent extracts of all trypanosomatids that were examined contained a cysteine peptidase with properties identical to those of the T.cruzi enzyme. The peptidase cleaves preferentially on the carboxyl side of arginine residues and, to a lesser extent, lysine residues. Its molecular mass, as determined electrophoretically, is essentially the same in all trypanosomatids. Organisms that express the peptidase include T.cruzi (all stages of the life cycle), every species of Leishmania tested, T.brucei, C.fasciculata, Endotrypanum, T.percae, T.rangeli, and T.dionisii. We have not been able to detect an equivalent peptidase activity in any of the non-trypanosomatids that we have examined.

O 438 A TANDEMLY REPEATED GENE FAMILY IN LEISHMANIA DONOVANI WHICH ENCODES AN ANTIGEN HOMOLOGOUS TO HEAT SHOCK PROTEIN 70 (hsp70). J.MacFarlane,M.L.Blaxter,R.P.Bishop, M.A.Miles,J.M.Kelly, Dept. of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, U.K.

L. donovani (HU3) antigen cDNA clones were isolated from a λgt11 expression library after screening with serum from an infected patient. **One clone corresponded to a 68 kD pro-**mastigote-specific antigen. Sequence analysis revealed homology to the hsp70 gene family of other species. Antibodies against the λgt11 fusion protein were present in c.60% of visceral leishmaniasis patients tested. Genes coding for this antigen were present on a single chromosome as a series of 3.7 Kb tandem repeats. Sequencing of L. donovani hsp70 genomic clones has identified the GGMP amino acid repeats found in the C-terminal coding region of other parasite Hsp70s and a long (c.1.0 Kb) **3'-untranslated region. No heat-**shock elements (HSEs) have been located. **The level of the promastigote-specific mRNA** was temperature-independent in the range 23-42°C. **Current work is concentrating on the** immunology, expression and cellular localisation of this antigen.

O 439 MULTIPLE DISTINCT GP63 GENES IN LEISHMANIA DONOVANI, Rhaiza Maingon, Lynne Elson, Julian Crampton, Marcel Hommel and Michael Chance, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, United Kingdom.

GP63 is the major surface glycoprotein in Leishmania. A number of different activities which include immunodominance, proteolysis and macrophage binding have been associated with this molecule. Recently it was reported that there are multiple genes coding for GP63 clustered in tandem repeats in the genome of L.major and L.donovani (Button et al., (1989). Mol. Biochem. Parasitol. 32: 271). The GP63 genes within the cluster were reported to be identical in their coding regions.

We have been studying the structure and expression of the L.donovani GP63. GP63 clones were isolated from a genomic library (strain LV9) constructed in gtwes B. Several clone classes were defined using a 5' probe derived from the GP63 DNA sequence from L.major and a 3' probe derived from a L.donovani GP63 cDNA clone. The two most abundant clones are L.donovani specific, both each map to two similar sized chromosomes and both contain a 3' telomer motif which is expressed. However both genes differ strikingly in their physical maps and interestingly in their expression. Partial DNA sequence of one of these genes around the common macrophage binding site shows little homology with the corresponding region in the GP63 gene in L.major. Current results regarding the complexity of the GP63 gene family in L.donovani will be discussed with respect to the use of this molecule in the development of serodiagnosis and vaccines against Leishmaniasis.

O 440 THE ACTIVE SITE OF THE LEISHMANIA DONOVANI SECRETED ACID PHOSPHATASE IS RESISTANT TO PROTEASE TREATMENTS, David J. Mallinson and Dennis M. Dwyer, Cell Biology and Immunology Section, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD 20892 Promastigotes of Leishmania donovani constitutively secrete a soluble acid phosphatase activity (S-AcP). This enzyme is also released by amastigotes within infected macrophages in vitro. It has been suggested that S-AcP may play a role in protecting both developmental forms within their respective hydrolytic environments. Therefore, it was of interest to determine whether various protease treatments had any effect on S-AcP.

Culture supernatants from mid-log phase promastigotes grown in chemically defined medium were used as a source of S-AcP activity. These were treated with a variety of endo- and exoproteases. The activities of these proteases were confirmed using azocasein. Trypsin, chymotrypsin, subtilisin, pronase, dispase and papain, even at concentrations up to 500 ug ml^{-1}, had no apparent effect on the enzymatic activity of S-AcP. In contrast, results of SDS-PAGE autoradiography showed that all of these proteases degraded metabolically labeled S-AcP. The cumulative results demonstrate that although the S-AcP molecule is hydrolyzed by these proteases, its active-site remains fully functional. This property could be of importance in parasite survival.

O 441 CLONING AND CHARACTERIZATION OF A GALACTOSE-INHIBITABLE SURFACE LECTIN OF *ENTAMOEBA HISTOLYTICA*. **Barbara J. Mann, Per Hagblom, Frances D. Gillin, and William A. Petri. Department of Medicine, Division of Infectious Disease, University of Virginia, Charlottesville, VA. 22908.** ***Entamoeba histolytica*** **kills host cells in a contact-dependent manner. The adherence of *E. histolytica* trophozoites to human colonic mucus, colonic epithelium, and other target cells is mediated by a galactose(Gal)/ N-acetyl-galactosamine(GalNAc)-inhibitable surface lectin. The purified Gal/GalNAc lectin competitively inhibits adherence to target cells *in vitro* and is the major antigen recognized by human immune sera. The purified Gal/GalNAc lectin has a molecular weight of 260 kDa on SDS-PAGE. Upon reduction with ß-mercaptoethanol, two subunits of 170 kDa (heavy), and 35 kDa (light), are observed. Sequence of the first 15 amino-terminal amino acids of the heavy subunit and 21 amino-terminal amino acids of the light subunit were determined by sequential Edman degradation. The light subunit exhibited microheterogeneity with conservative changes in the amino acid residues at positions 3, 5, 6 and 9, suggesting that at least at least 2 genes encode the light subunit.. Oligonucleotide probes to the amino termini were used to identify mRNAs for the heavy and light subunits on Northern blots. A genomic library was screened with oligonucleotide probes and partial genomic clones for the heavy and light subunits were identified. Full length genomic clones will be isolated from a limited genomic library. Cloning and sequencing the genes for the *E. histolytica* adherence lectin will be the first step in understanding its structure and function.**

O 442 METACYCLIC VSG GENE EXPRESSION IN AFRICAN TRYPANOSOMES. Matthews,K., Shiels,P., Graham,S. and Barry, J.D. Institute of Genetics and Wellcome Unit of Molecular Parasitology, University of Glasgow, Church Street, Glasgow G11 5JS, U.K. The metacyclic stage of African trypanosomes, in the tsetse fly, is the first stage at which a variant surface glycoprotein (VSG) coat is synthesised. The number of metacyclic variable antigen types (M-VATs) is restricted, forming no more than 27 of the several hundred coats which may be displayed by bloodstream parasites. Most bloodstream VSG genes are located as silent copies at chromosome internal positions and require duplication into a telomeric expression site for activation. This is often mediated by "70bp repeat" motifs found 5' to telomeric VSG gene copies. Once in an expression site the VSG gene seems to be cotranscribed with more than one expression site associated gene (ESAG) in a long polycistronic transcription unit. The M-VAT gene expression mechanism and genomic environment are distinct from those used for bloodstream VSG genes and all M-VAT genes examined are on the telomeres of the very largest chromosomes. We have begun characterisation of two M-VAT expression loci. We have compared them with each other and with bloodstream expression site features at the DNA and RNA level. One M-VSG gene environment is unusual with respect to both 5' "70bp repeat" motifs and ESAGs; the other locus is being investigated. Additionally, using a model line of trypanosomes, we have observed the specific mode used for VSG gene activation in metacyclic trypanosomes.

O 443 THE USE OF FUSION OF THE MALTOSE BINDING PROTEIN (MBP) TO FILARIAL PARAMYOSIN TO MAP EPITOPES RECOGNIZED BY O. VOLVULUS INFECTED HUMANS.
Ron Limberger*, Claude Maina*, Tom Nutman+, and Larry McReynolds*. New England Biolabs, 32 Tozer Rd., Beverly, MA 01915* and the Laboratory of Parasitic Diseases, National Institues of Health, Bethesda, MD 20215+.
The paramyosin gene of D. immitis has been cloned in the λ gt11 expression vector. Sequence of the cDNA insert (2,550 base pairs) showed one open reading frame with no prolines. The paramyosin sequence has a 32% protein homology with S. mansoni paramyosin and a 40% protein homology with C. elegans myosin. A gene fusion was made between the MBP of E. coli and paramyosin. This allowed paramyosin to be purified in one step on an amylose affinity resin. The cloned antigen was recognized by sera from Guatamelan patients infected with the filarial parasite Onchocerca volvulus. Mapping of the antigenic sites in paramyosin was done by the construction of six subclones of paramyosin fused to MBP. The sera from infected humans recognized discreet regions of the protein and these regions varied between patients. However, the epitopes did not change after the patients were treated with ivermectin. Paramyosin also stimulated significant T cell proliferation in 7 out of 16 of the infected patients. This approach should allow the identification of defined epitopes that induce the immune response in filarial infections.

O 444 ORGANIZATION OF THE GENE FAMILY ENCODING THE MAJOR SURFACE GLYCOPROTEIN (gp63) OF Leishmania mexicana mexicana. Enrique Medina-Acosta (1), David G. Russell (2), Roger E. Karess (3). Departments of (1) Medical and Molecular Parasitology, (2) Pathology and (3) Biochemistry. NYU Medical Center 550 First Avenue, New York, N.Y., 10016.
To study the regulation of the expression of gp63, the major surface glycoprotein of all pathogenic species of Leishmania, we have isolated and partially characterized gp63-related cDNAs. A lambda gt11 expression library was constructed using poly(A+)RNA from the promastigote form of Leishmania mexicana mexicana (Lmm). The library was screened by plaque hybridization with a Leishmania major (Ltm) genomic clone containing a complete gp63 ORF (1.8Kb) and both 5' and 3' untranslated regions (Button and McMaster J.Exp. Med. 167:724, 1988). Four cDNA clones, ranging in size from 5 to 2Kb, were characterized. While the physical maps resemble each other (and the Ltm genomic clone), there are restriction sites which are clone-specific (Diagnostic sites). As judged by Southern blot analysis of genomic DNA digested with diagnostic cutters, these cDNAs do not represent overlapping transcripts of a single transcriptional unit but rather correspond to bona fide transcripts of at least three distinct clusters of tandemly repeated gp63 genes. As in the case of Ltm, all of the gp63 genes of Lmm are linked on a chromosomal band of about 700Kb. An abundant 3Kb RNA species, hybridizing to the clones, is present in both promastigotes and amastigotes. The detailed genomic organization of the gp63 gene clusters as well as implications for protein expression are currently under investigation.

O 445 **DEVELOPMENTAL EXPRESSION OF THE GLYCOSYL-PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C OF *TRYPANOSOMA BRUCEI*.**

Kojo Mensa-Wilmot, Dale Hereld, Gerald W. Hart, and Paul T. Englund, Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205. The surface of the bloodstream form of the African Trypanosome is covered with about 10^7 copies of variant surface glycoprotein (VSG). VSG is tethered to the plasma membrane by a glycosyl-phosphatidylinositol (GPI) membrane anchor which is cleavable by an endogenous GPI-specific phospholipase C (GPI-PLC). Using a pleomorphic strain of *T. brucei*, we have examined the developmental expression of GPI-PLC. In agreement with Bulow and Overath [FEBS Lett. **187**, 105 (1985)], procyclic trypanosomes express about 0.1% enzyme activity as compared to bloodstream forms. Enzymological studies give no indication a diffusible inhibitor of GPI-PLC activity in procyclic forms. Northern blot analysis with a GPI-PLC-specific cDNA probe [(Hereld *et al.*, Proc. Natl. Acad. Sci. **85**, 8914 (1988)] reveals barely detectable levels of mRNA in procyclic forms. Hence, the lower amount of enyme activity in procyclics correlates with the mRNA level. Southern blot analysis is consistent with the presence of a single gene per haploid genome, and there is no evidence for rearrangement of the GPI-PLC gene after differentiation to procyclic forms which could potentially silence GPI-PLC expression. Western blot analysis reveals no detectable GPI-PLC polypeptide in procyclics (supported by NIH grant AI 21334 and by grants from the Rockefeller and MacArthur Foundations).

O 446 ERYTHROCYTE MEMBRANE PROTEINS IN THE PARASITOPHOROUS VACUOLAR MEMBRANE (PVM) OF P. FALCIPARUM, Ross B. Mikkelsen, Arun Singhal and Rupert Schmidt-Ullrich, Department of Radiation Oncology, Medical College of Virginia, Richmond, VA 23298.

The invasion of erythrocytes by merozoites represents a series of complex events culminating in the formation of the PVM. Previous studies from this lab demonstrated that the merozoite injects phospholipid into the host membrane, a necessary step for membrane expansion and formation of the PVM. We have now used specific antibodies and digitized immmunofluorescence microscopy to show that both band 3 and glycophorin are present in the PVM. Using both fixed and viable parasites free of the host membrane we also demonstrate that they retain their normal transmembrane orientation. With maturation, the amount of glycophorin in the PVM remains constant from early ring to schizont. In contrast, there is a progressive increase in the amount of band 3 in the PVM and a corresponding decrease in the host membrane with parasite development. This microscopic analysis was confirmed biochemically by the culture of parasites with ^{125}I-labeled erythrocytes, the isolation of host membrane and free parasites and determination of the relative amounts of band 3 and glycophorin following gel electrophoresis. The significance of the anion transporter, band 3, in the PVM for parasite growth is currently being examined.

O 447 CLONING OF THE gp63 GENE OF Leishmania donovani chagasi, Richard A. Miller, Marilyn Parsons, Steven G. Reed, Seattle Biomedical Research Institute, Seattle, WA 98109.

The predominant surface protein on promastigotes of human pathogenic Leishmania spp. is a 63 kD glycoprotein which has protease activity and which can function as a macrophage receptor. Two recombinant clones were selected from a genomic library of the new world parasite L. donovani chagasi using the gp63 gene of L. major, a parasite restricted to the old world (provided by Dr. W. R. McMaster). Genomic Southern analysis indicates that the gp63 gene of L. d. c. is highly homologous to sequences contained in L. mexicana amazonensis, L. major, and L. braziliensis guyanensis DNA, but not to sequences found in T. cruzi or T. brucei DNA. The L. d. c. genome contains at least 7 tandemly linked copies of the gp63 gene with a repeat unit length of about 3.0 kB. Northern analysis of RNA from promastigotes of L. b. braziliensis, L. major, L. m. a. and L. d. c. indicates that the major gp63 mRNA is 2.4 kB, but larger mRNAs were also detected. Partial DNA sequence analysis indicates > 90% homology both at the nucleotide and amino acid level with the gp63 sequence of L. major in the portions of the coding region sequenced thus far, including the amino terminus of the prepropeptide. The region of highest homology with L. major is the putative transmembrane domain at the carboxy terminus of the peptide: the final 24 predicted amino acid residues are identical. By comparison, the intergenic regions are generally less than 50% homologous. Expression of cloned gp63 may be useful for future immunologic studies, including work on serodiagnosis and vaccine development.

O 448 STRUCTURE AND EXPRESSION OF THE PROCYCLIC ACIDIC REPETITIVE PROTEIN GENES OF *TRYPANOSOMA BRUCEI*. *Michael R. Mowatt, Gregory S. Wisdom, and Christine E. Clayton. The Rockefeller University, 1230 York Avenue, New York, NY 10021 and *Laboratory of Parasitic Diseases, NIAID, Bethesda, MD 20892, USA. The procyclic acidic repetitive proteins (PARPs) of *Trypanosoma brucei* are developmentally regulated surface proteins encoded by a family of polymorphic genes. We have determined the complete nucleotide sequence of three members of the PARP gene family and investigated their expression. The amino acid sequence deduced from one novel member, the parpAα gene, showed a marked conservation of both the amino- and carboxy-terminal regions as compared to other PARPS, but revealed the substitution of a pentapeptide for the dipeptide repeating unit characteristic of all other PARPs. Northern hybridization analysis indicated that expression of the parpAα gene, like other members of this gene family, is confined to the procyclic stage of the *T. brucei* life cycle. This result implies coordinate regulation of the unlinked genetic loci that encode PARPs.

PARP was purified from cultured procyclics by cell fractionation followed by ion-exchange and Concanavalin-A sepharose affinity chromatography. PARP is membrane-bound and comprises about 1% of the total procyclic trypanosome protein or 6 x 10^6 molecules per parasite. The results of N-terminal sequencing and amino acid analysis indicate that PARP is processed by removal of both an N-terminal signal sequence and a hydrophobic C-terminal peptide. Metabolic labelling of PARP with [^{3}H]ethanolamine is consistent with attachment of the protein to the membrane via a glycosyl-phosphatidylinositol anchor.

O 449 SUBCELLULAR FRACTIONATION OF PLASMODIUM FALCIPARUM RHOPTRY ORGANELLES, Marianne C. Murray, Helen Shio, Margaret E. Perkins, Department of Biochemical Parasitology, The Rockefeller University, New York, New York 10021

The rhoptry is an organelle of the malarial merozoite, the extracellular stage of the erythrocytic cycle, which has been suggested to play an important role in the invasion of the erythrocyte. Although rhoptry proteins have been identified, their specific role in parasite invasion of the erythrocyte is unknown. The rhoptry organelles found in plasmodia and in other closely related members of the Apicomplexa have not been isolated to date. We have attempted to purify and characterize the rhoptry organelles from Plasmodium falciparum. It was found necessary to initiate the isolation from free merozoites as conditions necessary to rupture schizont-infected erythrocytes also disrupted the majority of the rhoptries. The rhoptries were enriched from merozoite homogenates by equilibrium density centrifugation in a gradient of sucrose. A separation of rhoptries from merozoite membranes and erythrocyte membranes was achieved in a linear sucrose gradient. Monoclonal antibody directed against the P. falciparum rhoptry protein of 110 Kd was used as a probe to detect fractions containing intact organelles. The density of P. falciparum rhoptries in sucrose was 1.19 g/ml. By electron microscopy, rhoptries were intact and their diameters were approximately 0.3-0.5 μm. Currently the properties of the rhoptry organelles are being investigated and this will be discussed.

Gene Expression; Membrane Molecules-II

O 450 TRANSCRIPTIONAL ANALYSIS OF THE MAJOR MEROZOITE SURFACE ANTIGEN PRECURSOR (GP195) GENE OF *PLASMODIUM FALCIPARUM*

Peter J. Myler, Seattle Biomedical Research Institute, 4 Nickerson St., Seattle, WA 98109-1651.
cDNA and genomic clones of the gp195 gene have been isolated from the Palo Alto PLF-3/B11 strain of *P.falciparum* and > 7 kb of nucleotide sequence obtained. Comparison of the amino acid sequence of the Palo Alto PLF-3/B11 gene with other gp195 genes showed close similarity to that of the Wellcome strain (1). Analysis of steady state RNA from different asexual blood stages of the parasite showed the presence of a 6.1 kb transcript which was significantly less abundant in ring stage RNA and most abundant in late trophozoite/early schizont stage RNA. This suggests that the stage-specific expression of the gp195 gene is regulated, at least in part, at the level of transcription. The 5' and 3' ends of the transcript have been mapped by primer extension and RNase protection experiments, in addition to sequence analysis of cDNA clones. The primer extension analyses identified several potential primer extension sites between 250 and 550 bp 5' to the ATG initiation codon. Potential TATA and CAAT box sequences have been identified upstream of these initiation sites. Preliminary evidence suggests that the more 5' transcription initiation sites are preferentially used in ring stages while the more 3' sites are used in trophozoite and schizont stages. These results have important implications for the mechanism(s) regulating stage-specific gene expression in *P.falciparum*.

1. Holder *et al, Nature* **317**: 270-273 (1985).

O 451 BIOCHEMICAL AND IMMUNOLOGICAL DISTINCTIONS BETWEEN P. falciparum ANTIGENS gp195 and EBA175, P.A. Orlandi, B.K.L. Sim, J.A. Lyon, J.D. Chulay, and J.D. Haynes, Department of Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20307. Two merozoite-associated antigens expressed during the later stages of intraerythrocytic development and schizont rupture include gp195, the major merozoite surface glycoprotein; and EBA175, a 175 kD erythrocyte binding antigen specific for sialic acid-containing determinants on the red cell surface. A recent study (Perkins and Rocco, J. Immunol., 1988, 141, 3190.) suggested a possible precursor-product relationship between Pf200 (gp195) and EBA175 found in the culture supernatants of rupturing schizonts based primarily on their ability to bind and interact with the red cell surface under similar conditions. In our investigations, mature schizonts were radiolabelled with isoleucine, methionine, and carbohydrate precursors and the cellular and supernatant fractions used in subsequent analyses. Partially purified EBA175 was isolated from these culture supernatants through incubation with human red cells followed by silicone oil separation and salt elution. Immunoprecipations with monoclonal antibodies against gp195 follwed by western blot analysis using an affinity-purified monospecific antibody reagent against EBA175 or anti-gp195 monoclonals indicated the two proteins in question are distinct without evidence of significant antibody cross-reactivity. EBA175 did not label with any carbohydrate moieties used whereas gp195 did. In IFA, antibodies against gp195 stained the surface of the merozoite uniformly, while antibodies against EBA175 stained the apical region only. (See Sim, et al.). Thus gp195 and EBA175 can be distinguished by their cellular location, reactivity with specific antibody reagents, as well as their molecular weights.

O 452 MOLECULES INVOLVED IN THE CONTACT OF Entamoeba histolytica WITH TARGET CELL. Orozco, E; Rodríguez, M.A; Hernández, F; Santos L; and Valdés, A. Department of Genetics and Molecular Biology. CINVESTAV-IPN. Apdo. Postal 14-740 México 07000, D.F. To detect molecules of Entamoeba histolytica involved in the trophozoite-target cell interaction, independent rabbit antisera against both total amebic proteins and 112 kDa adhesin were produced. Mouse antiserum against amebic molecules which adhere to the RBC surface after incubation of mouse RBCs with trophozoites was also generated (anti-adhesins). All three anti-sera recognized the 112 kDa adhesin. Adhesion of the 112 kDa adhesin on the RBC surface is temperature-dependent. At 37°C a high amount of it was found on RBCs and it remained there after washing with detergent. Different experiments using the anti-adhesins and the anti-total amebic proteins sera, revealed for the first time, that proteins of 90, 70, 50, and 24 kDa are involved in the contact of trophozoites to target cells. Adhesins of 210, 160 and 112 kDa molecular weight previously described were also detected in our experiments. Surface labeled MDCK cell proteins specifically adhered to 112, 90, and 48 to 50 kDa and other higher molecular weight amebic proteins, indicating that these molecules participate also in the contact of trophozoites to epithelial cells. Virulence-deficient mutants had the same amount of 112 kDa adhesin than the wild type strain. This adhesin, however, was not functional in mutants. Proteins of 90 and 24 kDa proteins also were altered in amebic virulence-deficient mutants and were recognized by the IgGs to 112 kDa adhesin, suggesting that they are specific breakdown products of the 112 kDa adhesin, or proteins sharing epitopes with this adhesin.

O 453 ISOLATION AND CHARACTERIZATION OF A cDNA CLONE ENCODING A MAJOR SURFACE ANTIGEN OF Giardia lamblia, Guadalupe Ortega-Pierres, Ramón Coral-Vázquez, Lourdes Cervantes, George Newport and Cecilia Montañez. Centro de Investigación y de Estudios Avanzados del IPN 07000, D.F. and University of California, Berkeley CA 94625. Recent studies from our group have suggested that surface antigens of Giardia lambia play a role in parasite attachment to epithelial cells. In particular, an antigen of approximately 65 Kd has been identified and seems to be a major surface component of this parasite. To further characterize this surface molecule, as well as to understand its expression, a recombinant clone from a G. lamblia cDNA library prepared in the expression vector λgtII was isolated using a rabbit sera prepared against the 65 Kd surface molecule. The fusion polypeptide expressed by the recombinant clone, upon induction with IPTG, has a molecular weight of approximately 140 Kd as determined by SDS-PAGE and Western blot analysis. Endonuclease digestion of the recombinant phage with EcoR1 revealed an 600 bp insert. The isolated DNA insert was hybridized to a G. lamblia DNA population of low density which was separated by CsCl centrifugation in the presence of the dye Hoescht 33258 and digested with various endonucleases. Hybridization to several bands was observed suggesting the presence of more than one gene encoding for the 65 Kd antigen. We are currently characterizing further the isolated clone by Northern analysis and sequencing determination.

O 454 ULTRASTRUCTURAL LOCALIZATION, GENE SEQUENCE, AND PREDICTED PROTEIN SEQUENCE OF A GIARDIN, A MAJOR CYTOSKELETAL PROTEIN OF GIARDIA LAMBLIA, Debra A. Peattie*, Rogelio A. Alonso*, Ann Hein+, and John P. Caulfield**, *Department of Tropical Public Health and Division of Biological Sciences, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115 and +Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115. Giardia lamblia, an anaerobic, flagellated protozoan, colonizes the upper intestine of vertebrates by virtue of a single, large sucking disc on its ventral surface. We have purified one of the giardins, a major group of cytoskeletal proteins and elemental components of the sucking disc, and determined its ultrastructural location via immunomicroscopy. We also have isolated and sequenced the gene for this giardin, allowing us to predict its complete protein sequence. The protein is 33,500 daltons molecular weight, has an abundance of helix-forming residues, and shares structural homologies with several calcium-binding proteins.

O 455 THE 11-1 GENE OF PLASMODIUM FALCIPARUM ENCODES A MEGADALTON PROTEIN, Carolyn Petersen, James Leech, Artur Scherf and Richard Nelson, The Medical Service, San Francisco General Hospital, San Francisco, CA 94110; Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143 and Institut Pasteur, Paris, Cedex 15, France. The 11-1 locus of Plasmodium falciparum, a genomic locus extending over 30 kb, contains tandem repeats of 9, 18 and 27 base pairs surrounded by unique sequences (Scherf et. al., EMBO, 1988). We attempted to identify the gene product of this locus using antibodies specific for the 27 bp repeat and a region containing both 9 and 18 repeats as well as human immune sera affinity purified on fusion proteins of genomic and cDNA inserts isolated by hybridization to the 27 bp repeat. Immunoprecipitation of metabolically labelled mature parasites with these antisera revealed a complex set of proteins including an approximately 1.5 megadalton protein, a >250 kD protein and a doublet at 140 kD. Antibody to the >250 kD protein was affinity purified from immune human serum.

Genomic Southern analysis using a 1.8 kD cDNA probe which encoded a portion of the >250 kD protein and a 27 bp oligonucleotide probe indicates that the >250 kD protein and the 1.5 mD protein are the products of separate genes. The 140 kD doublet comigrates with RESA which previously has been shown to be encoded by a different gene (Cowman et. al., Mol. Biol. Med., 1984). We conclude that the 1.5 megadalton protein is encoded by the 11-1 gene and contains epitopes which cross react with the >250 kD protein and RESA.

O 456 CALCIUM AS A POSSIBLE SECOND MESSENGER OF EXTERNAL SIGNALS IN Leishmania donovani, Philosoph, H. and Zilberstein, D., Department of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel. The identification of the host by the parasite and its subsequent adaptive mechanisms to its new environment might involve the transduction of signals from the host to the parasite. The possibility that calcium plays a role in mediating such signals was investigated in L. donovani promastigotes. By using the fluorescent Ca^{2+} indicator fura-2, we show that the concentration of free calcium in the cytoplasm of L. donovani promastigotes is maintained at very low levels (73.5±10 - 94±8 nM at $[Ca^{2+}]_o$ range of 0 - 1 mM). The maintenance of low $[Ca^{2+}]_i$ is energy dependent as it is disrupted by KCN, H^+-ATPase inhibitors, and ionophores. The low concentration of free calcium is maintained by intracellular pools as indicated using the metabolic inhibitors. Intracellular traffic of calcium was examined by measuring the transport of $^{45}Ca^{2+}$ in digitonin permeabilized promastigotes. Two transport systems for calcium were identified in these cells. One is respiration dependent, suggesting a mitochondrial localization. A second system is respiration independent but requires either endogenous or externally added ATP. The ATP-dependent Ca^{2+} transport is optimal at pH 7.1, has high affinity for calcium (Km=92 nM, Vmax=1 nmoles/min/mg protein) and is sensitive to orthovanadate. These properties suggest the presence of a Ca^{2+}-ATPase similar to that of mammalian endoplasmic reticulum. In preliminary experiments we found that GTP and inositol 1,4,5-trisphosphate, caused a release of calcium from the endoplasmic reticulum of digitonin permeabilized promastigotes.

O 457 A GROUP OF SMALL STRESS PROTEINS ARE EXPRESSED IN LEISHMANIA MEXICANA PARASITES DURING STAGE TRANSFORMATION, Elena Pinelli and Michal Shapira, Department of Chemical Immunology, MacArthur Center for Molecular Parasitology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Stage transformation of Leishmania parasites from promastigotes to amastigotes involves acquisition of thermotolerance, necessary for survival within the mammalian host. We have previously shown that stress proteins of 70 and 83 Kd which are induced in promastigotes exposed to elevated temperatures and stay constitutively expressed in amastigotes. Small HSPs vary extensively in structure and number among various species, ranging from one in yeast and mammalian cells, four structurally related proteins in Drosophila cells, to over 30 in plants. Leishmania parasites synthesize a set of small HSPs that are currently being studied. Analysis on 2-D gels of protein cell extracts metabolically labelled at 26°C and 37°C reveals a group of proteins with molecular weights ranging from 14 to 42 Kd, which are induced at 37°C. Several of these small stress proteins appear as isoforms. The 22 Kd and 28 Kd proteins are phosphorylated. Small HSPs in other organisms aggregate upon heat shock and concentrate in specific electron dense stress granules. Such granules are also observed in Leishmania amastigotes and heat shocked promastigotes. Further immunological analysis is currently underway.

O 458 TWO GENETICALLY DISTINCT MODES OF SURFACE ANTIGENIC VARIATION IN THE NEMATODE CAENORHABDITIS ELEGANS, Samuel M. Politz, Miguel Estevez, Peter J. O'Brien, and Karl J. Chin, Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609.

We have studied the genetic basis of nematode surface antigenicity, using the free-living species Caenorhabditis elegans as a model nematode. Rabbit antisera directed against the adult C. elegans cuticle were used in conjunction with formal genetic analysis of mutations to identify two distinct mutant surface antigen phenotypes. An antigenic polymorphism on chromosome II, designated srf-1, causes several C. elegans varietal strains to display antigen-negative phenotypes compared to wild-type. In contrast, EMS-induced mutations in two genes designated srf-2 and srf-3 cause surface expression of antigenic determinants not located on the wild-type surface. These and the results of differential cross-adsorption and epistasis experiments suggest that some mutations cause loss of surface antigenicity, while others, because of the layered arrangement of the cuticle, can cause gain of antigenicity by exposing surface antigens normally hidden in the wild-type cuticle.

O 459 BIOPHYSICAL PROPERTIES OF THE NEMATODE SURFACE: A FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING (FRAP) STUDY, Lorna Proudfoot, John R. Kusel, Malcom W. Kennedy and Huw V. Smith, Department of Biochemistry, University of Glasgow, G12 8QQ

Epicuticles of several nematodes studied could allow insertion of fluorescent lipid analogues for the measurement of lipid mobility. The biophysical technique of fluorescence recovery after photobleaching (FRAP), gave mobilities, of near zero for adults of *Brugia pahangi, Trichinella spiralis,* L_2- stage larvae of *Toxocara canis,* and dauer larvae of the free-living nematode *Caenorhabditis elegans.* However, indications are that proteins are free to move, as results from lectin diffusion and sulphydryl reagent diffusion show. Implications for the importance of lipid immobility in immune evasion may be far-reaching and current studies are designed to probe the nature of the nematode epicuticle.

O 460 EXPRESSION AND SITE-DIRECTED MUTAGENESIS OF A SCHISTOSOME SERINE PROTEASE, J.F. Railey, C.S. Craik, F.E. Cohen, L. Gregoret and J.H. McKerrow, Departments of Pathology and Pharmaceutical Chemistry, University of California, San Francisco, CA 94143

The protease secreted by cercariae of *Schistosoma mansoni* is required for the initial infection of the human host. This enzyme is an elastase and has regions of sequence homology to the trypsin family of serine proteases. Like pancreatic elastase II and chymotrypsin, the schistosome protease prefers substrates with aromatic amino acids at the P-1 subsite. A three-dimensional model has been generated based upon sequence similarities to other serine proteases. This model places the G_{210} residue at the S-1 binding pocket permitting a large aromatic side chain to be accommodated. To begin testing the predicted three-dimensional structure, the cercarial elastase was expressed using a bacterial plasmid vector (pTRAP) containing the cloned cDNA coding sequences fused with the DNA sequences encoding the signal peptide and regulatory regions for bacterial alkaline phosphatase. The G_{210} residue was changed to V_{210} by DNA site-directed mutagenesis, and this mutant enzyme was also expressed in *E. coli*. The substrate specificity of the mutant was altered so that the relative activity of the native enzyme for peptide substrates with phenylalanine at the P-1 subsite was reduced while activity to peptides with a smaller alanine residue at P-1 remain unchanged.

O 461 BIOCHEMICAL CHARACTERIZATION OF AXENICALLY GROWN LEISHMANIA AMASTIGOTES, Petrie M. Rainey, Terry Spithill and Alfred A. Pan, Yale University School of Medicine, New Haven, CT 06510.

Axenically grown amastigote-like forms of Leishmania mexicana pifanoi have been shown to closely resemble amastigotes isolated from infected mice or cultured macrophages in terms of kinetics of growth and transformation to promastigotes; rates of protein, RNA and DNA synthesis; rates of metabolism of glucose and fatty acids; proteinase, ribonuclease, adenine deaminase, alanine aminotransferase and peroxidase activity; and total protein patterns on SDS-PAGE. Promastigotes differed from both axenic amastigote-like forms and isolated amastigotes in terms of these characteristics. The developmentally regulated 35 kd protein homologous to aldose reductase and encoded by the P100/11E gene was found to be expressed in at least 100-fold greater concentration in promastigotes than in axenic amastigote-like forms or macrophage-derived amastigotes. Aldose reductase inhibitors block the growth of both promastigote and amastigote-like forms. These results suggest that axenically grown amastigote-like forms of L. m. pifanoi represent a valuable model for the study of Leishmania amastigotes.

O 462 CHARACTERIZATION OF A GENE ENCODING SUPEROXIDE DISMUTASE FROM SCHISTOSOMA MANSONI AND ITS PUTATIVE MEMBRANE-ASSOCIATED GENE PRODUCT

David M. Rekosh, Zhi Hong, Maryanne C. Simurda and Philip T. LoVerde, Departments of Biochemistry and Microbiology, S.U.N.Y. at Buffalo, Buffalo, N.Y. 14214.

The sequence of a mRNA encoding a Cu/Zn superoxide dismutase (SOD; E.C. 1.15.1.1) and the entire coding portion of its cognate gene has been determined. The gene spans 5.1kb of chromosomal DNA and possesses three exons and two introns which interrupt the coding region. The introns contain splice junction sequences which fit the consensus observed in mammalian genes. The upstream region contains elements characteristic of a promoter and the downstream region contains two potential polyadenylation sites. The deduced polypeptide sequence shows a 40% to 45% homology with the cytosolic superoxide dismutase from 10 other species and also a striking homology to the human extracellular form of the enzyme in that the sequence contains a hydrophobic leader and potential glycosylation site. This suggests that the SOD might be membrane-associated or secreted. Indeed, fractionation of schistosomula, juvenile and adult worms indicates that the majority of the SOD activity is present in NP-40 extractable and extracellular fractions. Isoelectric focusing gel electrophoresis of the Cu/Zn SOD activities present in the different stages, reveals varying patterns of bands, suggesting modification of the enzyme in different stages of the life cycle. Future work will focus on the possible role of surface-associated SOD in immune evasion.

O 463 CHARACTERIZATION OF A FAMILY OF TRANSCRIPTS ENCODING THE 31kDa MAJOR EGGSHELL COMPONENT OF FASCIOLA HEPATICA. Allison C. Rice-Ficht*, Kathryn A. Dusek* and J. Herbert Waite+, Department of Medical Biochemistry and Genetics, Texas A&M University, College Station, TX 77843* and College of Marine Studies, University of Delaware, Lewes, DE 19958+

Fasciola hepatica eggshell production is based on a quinone-tanning process which involves cross-linking of a number of proteins containing dihydroxyphenylalanine (DOPA) residues. The major DOPA-containing protein has been purified to homogeneity and peptide sequence of several tryptic fragments has been obtained (Waite, J.H. and Rice-Ficht, A.C. [1987] Biochemistry 26, 7819). We report here the use of antibody directed against the highly purified protein (homogeneous in electrophoretic mobility and isoelectric point) to isolate two related cDNAs encoding 31kDa proteins. The cDNAs were isolated from an adult Fasciola hepatica expression library, sequenced and found to encode the predicted peptides. Hybridization analysis of genomic DNA under high stringency conditions indicates the presence of six related copies of the gene; the possible transcription of additional genes in the adult stage is under investigation.

O 464 RAPID CHANGES IN THE EXPRESSION OF A GENE ENCODING A CALCIUM BINDING PROTEIN DURING SCHISTOSOME METAMORPHOSIS. Israel Schechter, Daniela Ram, Zahava Grossman and Alexander Markovics, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

The cDNA and genomic clones encoding a calcium binding protein (CaBP) obtained from cercaria were sequenced. The encoded protein (69 amino acids long) shows clear resemblance to the domain structure and organization of CaBP molecules. The schistosome CaBP contains two typical calcium binding loops and it shows Ca^{++}-dependent electrophoretic mobility (increased with Ca^{++}-ions and decreased with EGTA). Northern blots revealed expression of the CaBP gene in cercaria but not in sporocyst, 24 hr somula or worm. The structure of the gene is similar to that of other eukaryotes and one intron interrupts the coding sequence. The region of the cap site was determined and there was no evidence for spliced leader sequence. The CaBP is interesting because most of the metabolic (e.g. activation of kinases) and physiological (e.g. contraction) events triggered by calcium ions are mediated via CaBP molecules. The preferential expression of this CaBP in cercaria probably reflects adaptation to this developmental stage, and prompts us to investigate what function(s) specific to cercaria it regulates. A general issue revealed by the CaBP is the rapid change in gene expression during schistosome metamorphosis. The CaBP mRNA is missing in the parasite residing in infected snail, but is readily detected in cercaria one hour after shedding. We identified other genes which are turned on or shut off within the short time interval (~1hr) of transition from snail to free swimming cercaria. This system is likely to provide information on the mechanism of stage - specific gene activation/inactivation, and on the molecular basis of host-parasite relationship.

O 465 **ISOLATION OF CALMODULIN RESPONSE ELEMENTS FROM TRYPANOSOMES USING RECOMBINANT TRYPANOSOME CALMODULIN (rTCaM).** S.A.Schleck, C.E.Egwuagu, and C.L.Patton. Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT 06510. Calmodulin (CaM), a highly conserved Ca^{++}-binding protein, has been isolated and characterized from several species of trypanosomes. CaM /Ca^{++} regulates the activation and suppression of several enzymes directly or indirectly via other response elements. CaM also regulates microtubule assembly/disassembly cycles, cell motility, glycogen metabolism, and Ca^{++} signal transduction. Trypanosomes express different levels of TCaM during their developmental cycle, but until now, no CaM response element has been isolated and characterized due primarily to the low yield of native TCaM (nTCaM). We have cloned, sequenced, and expressed *T.b.rhodesiense* CaM genes in *E. coli* to yield quantities sufficient for detailed studies. TCaM has 18 amino acid substitutions compared to vertebrate CaM. rTCaM and nTCaM exhibit the same M_r (16.5 kDa) and Ca^{++} mobility shift on SDS-PAGE analyses and are antigenically identical in Western Blots using polyclonal antibodies to either protein. Both activate cAMP-phosphodiesterase in a Ca^{++} dependent manner and display absorption maxima at 253, 259, 265, and 268.5 nm, similar to those reported for other CaMs. The spectra of rTCaM are altered by molar additions of Ca^{++} and reversed by EGTA. rTCaM coupled to Affi-Prep 10 (Bio-Rad) was used to FPLC/affinity purify TCaM/Ca^{++}-binding proteins from a cytosolic fraction of bloodstream trypanosomes (specifically a DEAE anionic-exchange 0-300mM salt eluate). The EGTA-eluted major polypeptides exhibited subunit molecular weights of 37, 50-52, 56, 60-65, and 76kDa; the 60-65kDa protein has been further purified on a MonoQ column and partially sequenced. Biotinylated rTCaM (BioTCaM) binds to these polypeptides in a Ca^{++}-dependent association on Western Blots. In addition BioTCaM binds proteins associated with the membrane/flagella pellet fraction, especially a 55-60kDa protein. Surface ^{125}I-labeled trypanosome proteins were also bound to a rTCaM affinity matrix. Taken together, these data support the presence of cytosolic and membrane or flagella pocket associated TCaM binding proteins. A λgt11 expression library of the trypomastigote stage of *T. cruzi* was screened with BioTCaM; 6 clones out of 10^6 plaques are undergoing amplification preparatory to sequence analyses.

O 466 HOST CELL MEMBRANE ALTERATIONS DURING INFECTION WITH PLASMODIUM FALCIPARUM (PF), A LASER RAMAN STUDY, Rupert schmidt-Ullrich, A. Singhal, S.P. Verma* and R.B. Mikkelsen, Departments Radiation Oncology, Medical College of Virginia, VCU, Richmond, VA 23298 and New England Medical Center, Boston, MA 02111

We have shown that the invasion of Pf merozoites into human red blood cells (RBC) is associated with a transfer of parasite phospholipids into the RBC and a shuttling of intrinsic RBC membrane proteins into the parasitophorous vacuolar membrane (PVM). We have now used Raman spectroscopy to examine the molecular changes in the RBC membrane in ring- and schizont-infected RBC. We have measured the CH-stretching region at 2700 to 3000 cm^{-1} of RBC membranes purified from parasitized cells. Plots of the intensity (I) of 2930 cm^{-1} (methyl CH-stretching) vs. the thermally stable 2850 cm^{-1} (symmetric methylenic CH-stretching) reveals a sharp transition at 39°C for normal RBC. In contrast, this transition decreases to 7°C and 2°C for ring- and schizont-infected RBC respectively indicating that a major change in the physical state of the RBC membrane occurs shortly after invasion of the parasite. These data are most compatible with a fluidization of the RBC membrane and a decreased lipid-protein interaction secondary to unfolding of the major intrinsic membrane proteins or removal of those components from the RBC membrane.

O 467 IDENTIFICATION AND CHARACTERIZATION OF A cDNA ENCODING AN ANTIGEN ASSOCIATED WITH THE SURFACE OF ONCHOCERCA VOLVULUS MICROFIALRIAE, Alan L. Scott and Jonathan D. Dinman, Department of Immunology and Infectious Diseases, Johns Hopkins University, Baltimore, MD 21205.

Lactoperoxidase-catalyzed labeling of surface-associated peptides was employed to demonstrate that O. volvulus microfilariae have a limited repertoire of surface-associated peptides. The major labeled proteins have apparent molecular weights of 18 and 14.5 kDa. Antibodies were raised in a rabbit that recognize the 18 kDa surface-associated peptide and bind to the surface of O. volvulus microfialriae. These antibodies were used to immunologically screen lambda gt11 cDNA libraries. One of the immunologically reactive recombinant clones, M2f.e, contained an insert of 1150 bp with a 494 bp open reading frame coding for a protein of 165 amino acids. The parasite protein produced by this clone had a predicted size of 18.1 kDa and was designated Ovms18. M2f.e was cloned into the expression vector pATH 3 for the production of a Ovms18/Trp E fusion protein. The Ovms18/Trp E fusion protein was partially purified and used to produce antibodies. The anti-Ovms18/Trp E antibodies recognized the surface of O. volvulus microfilariae and was immunologically reactive with an 18 kDa peptide associated with the surface of the microfiariae. The results from slot blot analysis using the coding sequence from M2f.e to probe the DNA from a large number of different nematode species indicate that M2f.e-like sequences occur in the genome of other filarial species.

O 468 A FAMILY OF HIGHLY REPETITIVE PROTEINS FROM THE MEMBRANE SKELETON OF TRYPANOSOMA BRUCEI. Thomas Seebeck, Andrew Hemphill, Marianne Affolter and *) Durward Lawson. Department of General Microbiology, University of Bern, CH-3012 BERN, Switzerland, and *) Department of Zoology, University College, LONDON WC1 E6BT, England.

The membrane skeleton of Trypanosoma brucei contains a family of large and highly repetitive proteins, which are associated with the subpellicular microtubules. They are confined to the microtubule-membrane complex of the cell body, and they are absent from the flagellum. Analysis of two members of this family has revealed that both proteins consist essentially of a large number of strongly conserved repeats of 38 amino acids length. Despite the identical length of their repeat units, the two proteins are clearly distinct in that they are coded for by different genes, and in that the amino acid sequence of their respective repeat units are different, though related (50 % identity).

Sequence comparisons lead to the unexpected finding that the sequences of both proteins are similar (42 and 61 % identity, respectively) to that of a recently described antigen from Trypanosoma cruzi (Ibanez et al., Mol.Biochem.Parasitol. 30, 1988, 27), which is strongly immunogenic in chronically infected Chagas patients. The functional role of these repetitive proteins in the membrane skeleton of T. brucei, as well as their potential role in the immunology of African trypanosomiasis are currently under investigation.

O 469 TUBULIN GENE(S) OF P. FALCIPARUM: ISOLATION AND CHARACTERIZATION

Kunal Sen and G. Nigel Godson,Department of Biochemistry, NYU Medical Center, 550 First Avenue,New York, New York 10016 Human malaria parasite Plasmodium falciparum has a complex life cycle which is likely to demand regulation of its cytoskeletal proteins. As tubulin is the most important cytoskeletal protein, we are attempting to isolate and characterize that gene system in P. falciparum. We screened a P. fal genomic DNA library with an oligonucleotide probe synthesized corresponding to amino acid residues 401 to 412 of chicken β tubulin using an AT bias codon calculated from P. falciparum published sequences. We isolated a 1642 bp long β tubulin gene (incomplete) starting from amino acid residue 59 compared to β tubulin of chicken embryo and containing the 3' non coding sequence. This gene also contains one 162 bp long intron at amino acid 350 and 88% homology was found at amino acid level with β chicken tubulin. We also screened a cDNA library using the same oligonucleotide probe and isolated one β tubulin and two α-tubulin genes. The β tubulin cDNA is 1558 n.t. and incomplete.Its sequence confirms the intron positions and 3' end. Our attempt to locate 5' end of β tubulin gene on the same DNA fragment has so far been unsuccessful. Southern blot analysis using α and β tubulin probes indicate that α and β tubulin genes in P. fal are unlinked that there are more than one β-tubulin genes. α and β tubulins appear to have three different size transcripts on Northern blots of total blood stage parasite RNA.

O 470 HSP83 OF LEISHMANIA MEXICANA AMAZONENSIS IS AN ABUNDANT CYTOPLASMIC PROTEIN WITH A TANDEMLY REPEATED GENOMIC ARRANGEMENT Michal Shapira, Department of Chemical Immunology, MacArthur Center for Molecular Parasitology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Antibodies against the high molecular weight heat shock proteins, HSPs 70 and 83, were obtained by immunization with synthetic peptides derived from conserved regions. Antibodies were raised against a synthetically prepared fragment which corresponds to the amino terminus of HSP 70 and were reactive with the denatured form of this protein. These antibodies did not react with the native form of the molecule. Antibodies to a synthetic peptide derived from a conserved fragment of HSP 83 recognized both the denatured and the native form of the molecule. HSP 83 is a highly abundant protein in Leishmania amastigotes and promastigotes exposed to elevated temperatures. Immunofluorescence analysis of heat shocked promastigotes of Leishmania mexicana amazonensis using the antipeptide antibodies to HSP 83 shows that this protein is distributed in the cytoplasm. Molecular characterization of the gene that codes for HSP 83 shows it is present in several copies of approximately 4 Kb arranged in tandem repeats.

O 471 INDUCTION OF STAGE SPECIFIC GENE EXPRESSION DURING LIFE CYCLE DEVELOPMENT IN THEILERIA ANNULATA B. Shiels, J. Glascodine, R. Hall, D. Brown and A. Tait. Wellcome Unit of Molecular Parasitology, University of Glasgow, Scotland, U.K.

To study the regulation of the gene expression of Theileria annulata as the organism differentiates through its life cycle, a series of monoclonal antibodies have been produced. Sets of monoclonals specifically react with each of the major stages in the bovine host: the sporozoite, the macroschizont and the piroplasm. Differentiation from the macroschizont to the (intermediate) microschizont stage can be induced in vitro by incubation of infected lymphoblastoid cells at increased temperature. This differentiation results in the expression of piroplasm monoclonal antibody epitopes and in the loss of macroschizont specific epitopes. One of the piroplasm antibodies (5E1) detects a 30kd polypeptide on western blots and we have shown that this polypeptide is expressed following heat induced differentiation. Using the 5E1 antibody we have isolated a clone from a T. annulata lambda gt11 library. a 3 kilobase EcoR1 insert from this clone detects 2 parasite genes on a genomic southern blot and two major transcripts in piroplasm RNA. Hybridisation of this clone to RNA from the different parasite stages indicates tht the stage specific expression of the gene(s) is regulated at the RNA level.

O 472 FURTHER CHARACTERIZATION OF THE GENE ENCODING EBA-175, AND INITIAL IMMUNOGENICITY STUDIES, B. Kim Lee Sim, Francis W. Klotz, J. Mark Carter, Palmer A. Orlandi, Jeffrey D. Chulay and J. David Haynes, Walter Reed Army Institute of Research, Washington D.C. 20307-5100 and Johns Hopkins School of Public Health, Baltimore, MD 21205. We have recently cloned and sequenced 3.9 kb of the gene encoding a 175 kD P. falciparum erythrocyte binding antigen (EBA 175), thought to act as a receptor in merozoite invasion of erythrocytes. The gene has an RNA transcript of about 7 kb suggesting that the 175 kD protein may represent a processed product of a larger protein. The deduced amino acid sequence of EBA 175 has no significant homology with other characterized P. falciparum antigens. We have been able to resolve 6 and 7 chromosome-sized fragments of the Camp and FCR strains respectively of P. falciparum. In both cases the EBA gene apparently resides on the largest fragment. A 31 amino acid peptide designated Peptide 2, and a 43 amino acid peptide designated Peptide 4 derived from the deduced amino acid sequence of EBA 175, were predicted to have high antigenicity. These peptides were synthesized, separately conjugated to KLH, and used to immunize BALB/c mice. These mice produced high levels of antibodies to the immunizing peptide. Antibodies to peptide 2 did not react with parasite antigens. Antibodies made to Peptide 4 recognized EBA-175 in Western blots and the apical region of P. falciparum merozoites in IFA. Preliminary experiments indicate that antibodies to Peptide 4 block merozoite invasion of erythrocytes. These findings demonstrate that EBA 175 is a unique protein with significant potential as a malaria vaccine candidate.

O 473 THE CLONING AND ANALYSIS OF GENES ENCODING MAJOR ANTIGENIC POLYPEPTIDES ASSOCIATED WITH THE TEGUMENTAL SURFACE MEMBRANE OF SCHISTOSOMA MANSONI, Andrew J. G. Simpson, Helen Meadows, Simon Jeffs, Paul Hagan, Raymond Allen, Pirlanta Omer Ali and S. Ronald Smithers, Division of Parasitology, The National Institute for Medical Research, Mill Hill, London, NW7 1AA, U.K.

The tegumental surface membrane of adult S. mansoni provides a protective barrier against immune effector mechanisms and also serves as an important absorptive surface. In addition, we have recently shown that antigens associated with the surface membrane can stimulate significant levels of protective immunity. In the CBA/Ca mouse, vaccination with isolated adult membranes has achieved protection of up to 40%, which is >85% of that stimulated by multiple vaccination with highly irradiated cercariae. Immunoprecipitation and immunoblotting analyses have demonstrated major antigens of M_r 32, 25, 22, 20, 15 and 8K associated with isolated surface membranes, cDNA clones encoding the M_r 25, 22, 15 and 8K antigens have now been isolated and sequenced. The cloned DNA as well as antibody specific to the expressed gene products have been used to examine basic structural features of the antigens, their presence in the major life cycle stages and their possible involvement in stimulating protective immunity.

O 474 ESAG1 GENES IN *TRYPANOSOMA BRUCEI*: A COMPARISON OF GENOMIC AND cDNA CLONES, Bob L. Smiley, John K. Scholler and Kenneth Stuart, Seattle Biomedical Research Institute, 4 Nickerson St., Seattle, WA 98109

ESAG1 homologous genes have been found in all *T. brucei* expression sites so far examined. RNA has also been detected by Northern hybridization which is homologous to an ESAG1 probe in all bloodstream stage VATs but not in procyclic stage. The RNA can be shown to be VAT specific if a suitable oligonucleotide probe is used. A series of cDNAs from an IsTat 1.7 (VAT 7) cDNA library have been sequenced and compared to each other, and to genomic ESAG1 genes. Two specific events have been detected. One is the loss of the VAT 7 specific ESAG1 gene from the VAT 7 expression site. This occurred when the 7 VSG gene was gene converted in an antigenic switch to VAT A^7. The second is an apparent processing of ESAG1 RNA. An oligonucleotide which specifically hybridizes to RNA and to the plasmid from which the sequence was derived does not hybridize to genomic DNA with the same efficiency; although at low intensity, single bands in genomic Southerns can be identified. The oligonucleotide hybridizations have mapped the VAT 7 ESAG1 gene to the Bgl II site in the genomic map, a restriction site which is present in the cDNA clone, 10 kb from the 7 VSG gene. The genomic copy of the ESAG1 gene is currently being isolated to compare to the cDNA data. The other ESAG1 gene sequences will also be presented and compared.

O 475 HOMOLOGOUS CELL SURFACE PROTEINS OF BABESIA RODHAINI

David Snary, Michael A.Smith & Stephen C.Nicholls, Department of Molecular Biology Wellcome Biotech, Langley Court, Beckenham, Kent, BR3 3BS, U.K.

A group of four membrane proteins on B. rodhaini has been identified by monoclonal antibodies. These proteins contain both immunologically distinct and common epitopes. The genes for all four proteins have been cloned and two have been sequenced. The sequence and cloning data demonstrate that all four genes are tandemly arranged on the chromosome, and that they have probably arisen by gene duplication. Although DNA sequences at the 5' and 3' ends of the two genes, including the regions coding for the signal peptides and hydrophobic tails, are homologous, the majority of the sequences within the genes are divergent. The translated amino acid sequences reflect the differences present in the DNA sequences, although conservative substitutions in the amino acid sequences are common. Furthermore, secondary structure predictions based on the amino acid sequences predict that the overall conformations of the proteins are very similar. The presence of duplicated genes coding for proteins with similar structures but divergent sequences suggests that there may have been selective immunological pressure for mutation or change within these genes.

O 476 A DEVELOPMENTALLY REGULATED GENE OF LEISHMANIA MAJOR SHOWS HOMOLOGY TO A SUPERFAMILY OF REDUCTASE GENES, T.W. Spithill, G.Z. Kidane, J.M. Pettitt, P.J. Murray, T. Glaser and N. Samaras, The Walter and Eliza Hall Institute of Medical Research, Melbourne 3050, Australia.

The transformation of **Leishmania** from promastigote to amastigote is accompanied by changes in the expression of several proteins whose function may be necessary for parasite survival in the sandfly vector or mammalian host. To characterize these proteins, we have cloned cDNA sequences that vary in abundance during the life cycle of **L.major**. One sequence (P100/11E) encodes a 1.6kb poly A^+RNA whose abundance is markedly elevated in promastigotes of **L.major**. The P100/11E sequence is a single copy gene located on chromosome band 20 in **L.major** and is conserved in the genomes of other **Kinetoplastida**. Antisera raised against the P100/11E polypeptide detect a soluble protein of Mr=35,000 in **L.major** which is conserved in other **Leishmania** species. Immunogold electron microscopy reveals that this protein is localized to the cytoplasm of **L.major**. The P100/11E DNA sequence predicts an acidic polypeptide of Mr=32,000 which shows 40-46% similarity with the superfamily of reductase proteins including 2,5-diketo-D-gluconic acid reductase, aldose reductase, aldehyde reductase and ρ-crystallin. The **L.major** reductase sequence contains the IPKS motif located at the active site of aldose/aldehyde reductase. These results show that **L.major** expresses a reductase homologue as a developmentally regulated gene product in promastigotes. Inhibitors of human aldose reductase block promastigote growth in vitro suggesting that reductase function is obligatory for the growth of **L.major**.

O 477 DETECTION OF VARIABLE SURFACE GLYCOPROTEIN EXPRESSION SITE-SPECIFIC SEQUENCES USING RIBONUCLEASES. Andrew W. Stadnyk, John K. Scholler and Ken Stuart, Seattle Biomedical Research Institute, 4 Nickerson St., Seattle, WA, 98109.

Genes within the Variable Surface Glycoprotein (VSG) gene expression site of *T. brucei* are often repeated within the same expression site, other expression sites, and elsewhere in the genome. This complicates the identification of DNA sequences from a single VSG gene expression site. We have prepared a genomic DNA library from a single chromosome (M4) using pulse field gel (PFG)-purified DNA. Using two cloned probes from an expression site of a heterologous stock of *T. brucei*, we isolated expression site associated sequences from our library. The library clones hybridized to multiple fragments in Southern blots, all large chromosomes, and primarily to a 1.2 kb transcript in Northern blots. One clone hybridized to transcripts only in bloodstream form trypanosomes. Radiolabeled transcripts were prepared from this clone and hybridized with RNAs from trypanosomes transcribing various VSG gene expression sites. The homology between this clone and transcripts from different expression sites was determined using RNAse A and RNAse T1 digestion of the hybrids. Following RNAse treatment, multiple small hybrids were preserved with all RNAs, indicating there are blocks of homology between expressed copies of this sequence. A full length transcript, indicating perfect homology, was preserved only in our variant antigenic type 5, which expresses the 5 VSG gene on M4. We hope to confirm the expression site-specific origin of these genomic clones and map expression sites using this methodology.

O 478 KARYOTYPIC ANALYSIS OF SEVERAL CLONED LEISHMANIA SPECIES. Holly B. Steinkraus, Sigrid Panitz, Theodor Hanekamp, and Pamela J. Langer, Department of Molecular Biology, University of Wyoming, Laramie, WY 82071. Several Leishmania species have been subcloned by a method involving a combination of limiting dilution and plating on rabbit blood agar plates. The subclones were verified by species specific monoclonal antibodies and are being tested for infectivity. Chromosomes were separated by contour clamped homogeneous electric field gel electrophoresis using various pulse times. Chromosomal migration patterns of parental and subclone isolates were compared. These gels indicate no discernable differences between the chromosomal patterns of these isolates. The chromosomes were hybridized with a variety of probes to determine the chromosomal localization of specific genes. These probes are also being used to identify recombinant clones from gene libraries constructed from the subcloned Leishmania species.

O 479 CHARACTERISATION OF TWO TRYPOMASTIGOTE-SPECIFIC GENES IN T.CRUZI
Garry B.Takle and Alison J.Young. Department of Molecular Biology, Wellcome Biotech, Langley Court, Beckenham, Kent, BR3 2BS, U.K.

Trypomastigote (mammalian stage)-specific surface molecules are possible vaccine candidates in Chagas' disease. Starting from a bank of trypomastigote stage-specific genomic clones from Trypanosoma cruzi we have further investigated the two clones hybridising to the largest mRNAs. Clones Tt34 (mRNA 3.5kbp) and Tt21 (mRNA 4.0kbp) have been sequenced and expressed and antisera to the fusion proteins have been used to identify the respective parasite polypeptides by immunoprecipitation and Western blotting. Both Tt34 (85kDa) and Tt21 (165kDa) encode trypomastigote-specific surface proteins. From Southern blotting and primer extension experiments and the DNA sequences, we have determined and will be presenting data on the predicted protein structures, genomic organisations and arrangements of the 5' ends of the transcripts.

O 480 COMPOSITE VARIANT SURFACE GLYCOPROTEINE (VSG) GENES OF *T. EQUIPERDUM*, Genevieve Thon, Theo Baltz and Harvey Eisen, Department of Genetics, FHCRC Seattle, WA 98104.
During the course of an experimental infection of the Rabbit *T. equiperdum* expresses a large repertoire of VSGs in a loosely predictable order. In an attempt to determine whether a molecular mechanism is involved in setting the order of expression, we have analysed the structure of two VSG genes. The genes coding for VSG-20 and VSG-20(bis) are both products of duplicative transpositions. The two genes have been made by the combination of several basic copies: an identical 3' donor and non-related 5' donors. We have cloned and sequenced the two cDNAs and the basic copies. Surprisingly, all the basic copies involved in the formation of the expressed genes are pseudogenes. The relative positions of sequence similarities in their sequences seem to determine where the recombinations occur. This suggests that once a productive combination of silent genes has been made and is expressed, some sequence replacements involving different basic copies are more likely to occur than others. For example sequence replacements leading to a switch from VSG-20(bis) to VSG-20 are expected to be more frequent than the reverse. We propose that such rules partly account for the order of expression of the VSGs.

O 481 **TRANSLATIONAL ACTIVATION OF THE GCN4 mRNA OCCURS WHEN THE RATE OF TRANSLATIONAL INITIATION FOR MOST OTHER mRNAs IS DECREASED: IMPLICATIONS ON THE MECHANISM OF RIBOSOME REINITIATION.** **Dimitris Tzamarias, Irene Roussou and George Thireos. Institute of Molecular Biology & Biotechnology, Foundation of Research and Technology, Heraclion 711 10, Crete, Greece.**

We present evidence showing that the steady state translational activation of the GCN4 mRNA in yeast, is based upon an increase in the rate of ribosome reinitiation following translation of the 5' most proximal open reading frame located in its untranslated region. Such an increase is effected when the cellular amount of the GCN2 protein kinase is increased or when the function of the *GCD1* gene product is defective. Both conditions result in decreased steady state rates of overall protein synthesis. A dramatic but transient increase in the rate of GCN4 protein synthesis also occurs immediately after amino acids are removed from the growth medium and which is also based on increased ribosome reinitiation. This transient activation of *GCN4* mRNA translation coincides with a severe transient decrease in the rate of total cellular protein synthesis. Under all these conditions, the process that is affected is formation of 43S preinitiation complexes. These results reveal the existence of a coupling between this step in translational initiation and the mechanism of ribosome reinitiation, and suggest possible functions for the GCN2 and GCD1 gene products.

O 482 GLUCOSE UPTAKE BY TRYPANOSOMA BRUCEI, Teresita Munoz-Antonia*, Christian Tschudi*, Benjamin Spencer* and Elisabetta Ullu*+, MacArthur Center for Molecular Parasitology, *Department of Internal Medicine and +Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

In Trypanosoma brucei, the requirement of glucose and its metabolism vary in different stages of the life cycle. It is not known whether the uptake of glucose in bloodstream and procyclic trypanosomes is by similar or different specific transport mechanisms. The specific uptake of glucose in bloodstream trypanosomes has been documented, but no attempts have been made to study glucose uptake in procyclics or to identify the molecule(s) involved in the transport. Using rapid centrifugation through a medium of defined density as a mean to separate free from intracellular glucose, we have measured glucose uptake in trypanosomes and shown that procyclic trypanosomes can uptake glucose in a specific, time- and dose-dependent manner. This uptake is inhibited by sulphydryl inhibitors and cytochalasin B. These compounds are known inhibitors of carrier mediated glucose uptake suggesting the presence of a glucose transporter in procyclic trypanosomes. These results are supported by the isolation of trypanosome cDNA clones that cross-hybridize with yeast and mammalian glucose transporter DNA probes.

O 483 THE RODENT MALARIA PARASITE PLASMODIUM BERGHEI AS A MODELSYSTEM FOR THE STUDY OF GENE-EXPRESSION DURING GAMETOCYTOGENESIS, Alex van Belkum*, Chris Janse, Barend Mons and Huub Schellekens*; *TNO Primate Center, P.O. Box 5815, 2280 HV Rijswijk, The Netherlands; Laboratory of Parasitology, University of Leiden, P.O. Box 9605, 2300 RC Leiden, The Netherlands.

In recent years detailed studies on the cell-differentiation of P. berghei, both in vivo and in vitro, have led to the establishment of well defined conditions for synchronised development of this parasite. Together with the availability of strains which differ in gametocyte-production, studies on the molecular mechanisms underlying sexual differentiation can be initiated. cDNA libraries have been constructed from poly-A^+ RNA isolated from both a strain capable of forming sexual cells and from a strain deficient in this respect. Using a variety of cDNA subtraction strategies, we are currently trying to isolate genes involved in sexual differentiation. The P. berghei system also offers the possibility to study the differential expression of malarial genes in general. During synchronous cultivation, RNA fractions can be isolated from different stages of development of the various blood-stages of the rodent parasite. It is also possible to culture and purify the early mosquito stages in vitro. When different strains are used (i.e. differing in gametocyte-production), gene-expression can be analysed by Northern blotting. At present we are mainly interested in the expression of A- and C-type ribosomal RNAs (in collaboration with Drs. Waters and McCutchan, NIAID, Bethesda, USA), actin-1 and actin-2 expression and the expression of DNA polymerase-genes.

O 484 Fl-160: A Flagellar Protein of *Trypanosoma cruzi* with Epitopes Cross-reactive with Mammalian Nervous Tissue.

Wesley C. Van Voorhis and Harvey Eisen. Fred Hutchinson Cancer Research Center and University of WA Medical School, Div. of Infect. Dis., Seattle, WA. Chagas' disease, caused by *Trypanosoma cruzi*, is an excellent model for autoimmune disease induced by an infectious agent. Transfer of T cells, directed against cross-reactive ags of *T. cruzi* and nervous tissue, have been shown to reproduce pathology found in chronic Chagas' disease. We used recombinant DNA technology to characterize one of these cross-reactive ags (Fl-160). We have cloned DNA from *T. cruzi*, that expresses a protein corresponding to a 160 kD protein found on the surface of the trypanosome, overlying the flagellum. Antibodies to this protein cross-react with a 48 kD mammalian nervous tissue protein, found in sciatic nerve, brain, and myenteric plexi of gut. The myenteric plexi are destroyed by inflammatory infiltrates in Chagas' disease, leading to the characteristic megaesophagus and megacolon Chagas' disease pathology. Thus, this ag is a candidate ag for autoimmune mimicry leading to nervous tissue pathology.

O 485 CLONING AND EXPRESSION OF THE HYPOXANTHINE-GUANINE PHOSPHORIBOSYL-TRANSFERASE GENE FROM PLASMODIUM FALCIPARUM IN E. COLI, Geetha Vasanthakumar, and Richard L. Davis, Jr., Molecular Biology Section, Southern Research Institute, Birmingham, AL 35255. Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) plays a key role in salvaging preformed purines for P. falciparum which cannot synthesize purine nucleotides de novo. Since P. falciparum cannot synthesize purines de novo, development of specific inhibitors for this enzyme should be an effective method of chemotherapy. To provide sufficient quantities of the enzyme for detailed biochemical and crystallographic analysis, we have isolated the gene encoding P. falciparum HGPRT, subcloned into ptac85 expression vector and transformed into E. coli strain deficient for both de novo purine synthesis and guanine utilization (strain GP120). When this clone (pRD500) was grown in the presence of isopropyl-β-D-thiogalactopyranoside (IPTG), which induces the tac promoter of the expression vector, a protein of 26 kD was produced. The size of this protein is in agreement with the predicted molecular weight deduced from the HGPRT cDNA open reading frame. We have also demonstrated significant HGPRT activity in cell-free extracts of GP120/pRD500 cultures grown in minimal medium containing xanthine as the sole source of purines and IPTG.

This project was supported by BRSG SO7 RR05676 awarded by the BRSG Program, Division of Research Resources, NIH.

O 487 *LEISHMANIA* GENES ENCODING REPETITIVE PEPTIDE ANTIGENS, Anne E. Wallis and W. Robert McMaster, Department of Medical Genetics, University of British Columbia, Vancouver, Canada, V6T 1W5.

Leishmania genes that encode protein antigens containing regions of tandemly repeated peptide sequence were identified using antibodies against *Leishmania* membranes. *L. major* has two genes which contain multiple tandem copies of a 42 base pair repeat sequence which are followed by very similar 3' non-repetitive sequences. However, the 5' regions of these genes do not cross-hybridize. One gene, Gene 20.1, encodes an mRNA of 9.5 nucleotides in length and contains over 100 of the 42 bp repeat sequences followed by a 3' non-repeat region of 475 bp. The second gene, Gene 20.2, encodes an mRNA of 5.2 nucleotides and contains only 8 tandem repeats followed by a 3' non-repetitive region of 450 base pairs which has a high degree of sequence similarity with the 3' region in Gene 20.1. Antibodies against this repetitive sequence detect a *L. major* protein of 240,000 MW. Northern blot analysis indicates that the two mRNA's encoded by Gene 20.1 and Gene 20.2 are expressed in both *L. major* and *L. donovani* and that these two mRNA's are expressed during both stages of the Leishmania life cycle. The *L. donovani* gene homologous to Gene 20.2 contains 6 tandem repeats of the same 42 base pair sequence found in the *L. major* genes. Pulse field gel electrophoresis and Southern blot analysis indicate that all *Leishmania* species examined contain this 42 bp repeat sequence, whereas, other trypanosomatids such as, *Trypanosoma cruzi* or *Crithidia* do not. Many other protozoan parasites have antigens which contain repetitive peptide sequences, however, unlike the repetitive antigens in *Plasmodium*, the repeat sequences in these *Leishmania* proteins are conserved both within and between *Leishmania* species.

O 488 COMPARISON OF THE GENOMIC DNA SEQUENCE ENCODING THE CELL SURFACE GLYCOPROTEIN Gp63 OF *Leishmania major* AND *L. donovani*, John R. Webb, Linda L. Button and W. Robert McMaster, Department of Medical Genetics, University of British Columbia, Vancouver, Canada V6T 1W5

The major surface glycoprotein (gp63) of *Leishmania spp.* is known to be homologous between diverse species of *Leishmania* and is suspected of playing an integral role in infectivity and antigenicity during the course of parasitism. In order to identify regions of gp63 which may be of functional significance, the sequence of genomic DNA encoding gp63 from *L. major* and *L. donovani* is being compared to identify regions of homology. A λEMBL3 genomic library constructed with Sau3A-digested *L. donovani* DNA fragments and was screened by plaque hybridization using a DNA probe from the previously cloned and sequenced *L. major* gp63 gene. A 3 kb SalI fragment which had homology with the 5' and 3' untranslated regions as well as the coding region of *L. major* gp63 was identified and was chosen for further analysis. This fragment was subcloned into the SalI site of M13mp19 for single stranded sequencing of exonuclease III generated deletions, and into the SalI site of the plasmid sequencing vector pAA3.7x for double stranded sequencing of transposon generated deletions. Sequence data indicate a high degree of similarity including 3' coding and 3' non-coding regions. The sequence encoding the proposed receptor recognition tripeptide Arg(375)-Gly(376)-Asp(377) is maintained in both species as are the potential *N*-glycosylation sites.

O 489 A PLASMODIUM VIVAX PROTEIN WITH RECEPTOR-LIKE SPECIFICITY FOR THE HUMAN DUFFY BLOOD GROUP GLYCOPROTEIN, Wertheimer S.P. and Barnwell J.W., Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, N.Y. 10016.
The Duffy blood group glycoprotein is an essential ligand for a P. vivax receptor during the parasite invasion of human erythrocytes. We have recently demonstrated the importance of this receptor-ligand interaction by blocking the in vitro invasion of human erythrocytes with an anti-Duffy monoclonal antibody (Mab). We report here the identification of a P. vivax protein which binds with receptor-like specificity to the Duffy glycoprotein on the surface of human erythrocytes. This protein, which we have designated P. vivax Duffy Associating Protein-1 (PvDAP-1), has a Mr of 135 to 140 Kd. PvDAP-1 binds only to cells which are susceptible to invasion by P. vivax. The binding is abrogated by treating the erythrocytes with chymotrypsin but not with either trypsin or V-8 protease. Binding of PvDAP-1 to human erythrocytes is inhibited by both the anti-Duffy (Mab) and purified human Duffy glycoprotein. We are currently characterizing the nature of the binding of PvDAP-1 to the Duffy glycoprotein, developing PvDAP-1 specific Mab and cloning the gene for PvDAP-1. The essential role of the Duffy glycoprotein in the process of invasion of human erythrocytes by P. vivax suggests that DAP(s) will be important in understanding the mechanism of parasite invasion.

O 490 THE SHARING OF PROTEIN MOLECULES BETWEEN THE FRESHWATER SNAIL BULINUS AFRICANUS AND SELECTIVE SCHISTOSOME PARASITES, Corrie T. Wolmarans and Kenné N. de Kock, Department of Zoology, Potchefstroom University for CHE, Potchefstroom 2520, South Africa. The compatible and non-compatible phenomena between B. africanus and selective schistosome parasites were investigated. In this study it appeared that both S. mansoni(non-compatible with B. africanus) and S. haematobium (compatible) share proteins with this freshwater snail. The degree of sharing was studied by binding the surface teguments of different larval stages of these parasites to rabbit anti-snail antibodies followed by coupling to goat anti-rabbit-Fitc. The sharing of proteins between the snail and parasite stages developed in mice was studied by polyacrylamide electrophoresis followed by western blotts. The results of this study showed that selective proteins synthesized by both the schistosome parasites and B. africanus are shared by these organisms. The role of these proteins in protecting the compatible parasite and rendering it recognizable in the non-compatible situation is presently under investigation.

O 491 IDENTIFICATION AND CHARACTERIZATION OF SCHISTOSOME TROPOMYOSIN, Han Xu, Shohreh Miller, Harry van Keulen, David M. Rekosh and Philip T. LoVerde, Department of Microbiology, SUNY at Buffalo, Buffalo, NY 14214 Human chronic infection sera or vaccinated mouse sera identified an acidic group of protein spots of about 40 kD in NP-40 extracts of S. mansoni 3hr schistosomula when analysed on Western blots of 2D gels. These spots were used to immunize mice and rabbits. A cDNA clone (clone 1) from a S. mansoni adult worm pBR322 library was isolated by using cDNA probes made from size-fractionated mRNA. Clone 1 was identified as encoding the 40 kDa antigen by hybridization selection of its cognate mRNA which was translated in vitro and the protein product immunoprecipitated with the specific mouse antiserum. By using the clone 1 insert as a probe, a lambda gt 11 expression clone which contained an insert close to the full size of the mRNA was isolated from a S. mansoni cercariae library. The complete sequence of the mRNA was determined by sequencing this clone and the mRNA. There was only one open reading frame in the 1,316 nucleotide sequence coding for a 284 amino acid polypeptide which showed homology (44.76% to 55.44%) with the amino acid sequence of eighteen different tropomyosins from various species. Northern analysis showed the mRNA was about 1.5 kb in size, and that the amount of detectable mRNA was much higher in the adult worm stage as compared to the cercariae and the egg stages. Hybridization patterns obtained by genomic Southern analysis of restriction digests suggest the existence of introns and/or multiple gene copies. Schistosome and chicken gizzard tropomyosin do not immunologically cross react as shown by Western blot analysis. (Supported by AI18867)

O 492 EXPRESSION OF SCHISTOSOMA JAPONICUM ANTIGENS IN E.coli Zheng Zhaoxin,Yan Weiyue,*Liu Shuxin,*Wu Gongze,*Tao yi-wen,*Xu Yuxing.,Fudan University,Shanghai,China.,*Institute of Parasitic Disease,Chinese Academy of Preventiv Medicine,Shanghai, China.,We isolated two clones which contain genes encoding Schiz-tosoma japonicum protein recognized by immune rabbit antisera . The genomic DNA library of S.japonicum was prepared from adult worms.Following the addition of EcoRI linker,the DNA was fract-ionated into 1.5-7.0 kb. The linked DNA was inserted into the EcoRI site of the β-galactosidase gene in λgt11.Screening the library with hyperimmune rabbit sera against S.japonicum.Of the 150000 plaques screened,only two clones reacted with the rabbit antiserum.The insert size was found to be 2.0kb.Western blot analysis of fusion proteins showed that the clone was producing hybrid proteins larger than native β-galactosidase.Rabbit antisera reacted with fusion protein but not with lysates of λgt11 infected bacteria.

O 493 REGULATION OF INTRACELLULAR pH BY PROTON PUMPS IN Leishmania, Zilberstein D. and Gepstein A., Department of Biology, Technion - Israel Institute of Technology, Haifa 32000, Israel. Promastigotes of L. donovani possess active transport of glucose and amino acids which are driven by proton motive force i.e. a proton electrochemical gradient is created across the parasite plasma membrane which is coupled to transport systems by maintaining symport translocation of the specific substrate with proton. We discovered that a proton translocating ATPase is located at the cytoplasmic surface of this parasite plasma membrane. This enzyme shows optimal activity at pH 6.5, is magnesium dependent, and is sensitive to orthovanadate. Our results indicate that this enzyme belongs to the P type ATPase and it is the primary proton pump in this organism. The role of this proton pump in regulating intracellular pH (pHi) in L. donovani promastigotes was investigated. Three methods were used for determining pHi in this organism: (a) Direct measurement of the pHi in triton X-100 cells extract, (b) Fluorescence pH indicator bis(carboxyethyl)carboxyfluorescein, and (c) Fluorescence amine acridine orange. L. donovani promastigotes regulate their pHi at 6.5 in the range of 5.0 - 7.4 pHo. The regulation of pHi is sodium-independent, but requires potassium in the external medium. The growth rate (12 hrs, at pHo 6-7) of promastigotes is related to the pHi (at 6.5) and not to the extracellular pH. Interestingly, both proline transport and protein synthesis show a similar pH dependence as the growth rate. Our overall results indicate that pHi in L. donovani promastigotes is highly regulated and that the plasma membrane H^+-ATPase plays a critical role in maintaining pHi and proton motive force.

O 494 IDENTIFICATION OF A FASCIOLA HEPATICA EGGSHELL PROTEIN AND TISSUE LOCALIZATION OF THE mRNA BY IN SITU HYBRIDIZATION. Mario Zurita*, David Bieber and Tag E. Mansour, Department of Pharmacology, Stanford University, Stanford, CA 94305. *CEINGEBI, UNAM/Mexico.

A cDNA clone, F3, isolated from a library constructed with RNA from the female genital complex of the liver fluke Fasciola hepatica has been reported (1). This clone encodes a 197 amino acid protein rich in Gly, Try and Lys. The F3 clone has been expressed in E. coli as a fusion protein with β-galactosidase. Using polyclonal antibodies raised against the recombinant protein, we were able to identify specific proteins present in adult, non-embryonated eggs and egg shell preparations. The proteins identified in the adult were 31 and 30 Kd; those in the egg and egg shells were 30 and 28 Kd. Using in situ hybridization the F3 RNA was detected in the vitelline follicles of the adult. These data demonstrate that the F3 clone codes for an eggshell protein that is synthesized as a precursor in the immature vitelline cells which line the vitelline follicle. The protien is processed to a mature product as the vitelline cells mature and contribute to the formation of the egg. A genomic clone has been isolated and the preliminary analysis of the 5' region of the gene is presented. (Supported by grants from the MacArthur Foundation and NIH.)

1. Zurita, M., Bieber, D., Ringold, G. and Mansour, T.E. (1987) PNAS 84:2340.

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O 500 IRON AND LIPID ACQUISITION FOR GROWTH AND MULTIPLICATION FOLLOW SPECIFIC *TRICHOMONAS VAGINALIS* BINDING AND LYSIS OF HUMAN ERYTHROCYTES. J.F. Alderete, D.C. Dailey, T.H. Chang and M.L. Lehker, Department of Microbiology, The University of Texas Health Science Center, San Antonio, TX 78284-7758. *Trichomonas vaginalis* is a human sexually-transmitted parasite which must obtain lipids and iron from the urogenital tract for growth and multiplication. These nutrients are acquired by specific, receptor-mediated mechanisms, such as the recognition and binding by *T. vaginalis* of human erythrocytes. Nonpathogenic human trichomonads, are incapable of erythrocyte attachment, indicating that adherence of red blood cells is related to overall *T. vaginalis* virulence. Metabolic integrity of the trichomonads is required for erythrocyte binding, which is also time, temperature, and pH dependent. At least two surface proteins of *T. vaginalis* have been identified as putative red blood cell adhesins. Hemolysis occurred in a contact-dependent fashion after erythrocyte attachment to trichomonads. Human red blood cells were able to provide lipids and hemoglobin-iron, which allowed for trichomonal growth and multiplication. This parasite maintenance resulted during nutrient-limiting conditions which do not normally allow for survival of *T. vaginalis* organisms. These observations are significant in terms of the availability and use of erythrocyte components for growth and multiplication as well as the establishment of infection and development of trichomoniasis.

O 501 AN UNUSUAL CYSTEINE PEPTIDASE OF TRYPANOSOMA CRUZI. Frank Ashall, Imperial College, South Kensington, London, U.K.

A cysteine peptidase was isolated from Trypanosoma cruzi and was shown to cleave bonds involving the carboxyl group of arginine and, to a lesser degree, lysine residues. The enzyme cleaves short peptide derivatives more readily than longer ones, but it is not simply an aminopeptidase. Examination of a range of synthetic peptide substrates demonstrated that the peptidase cleaves different substrates at quite different rates even it has the same Km values for the different substartes. The data are interpreted to mean that the enzyme binds the substrates with similar affinities but cleaves them at different rates once they are bound. The peptidase was detected in all stages of the life cycle of T.cruzi and has also been found in every other trypanosomatid that we have examined. We are collaborating on the development of specific inhibitors of the enzyme with a view to design of chemotherapeutic drugs for trypanosomatid diseases.

O 502 ENTAMOEBA HISTOLYTICA: PHYSIOLOGY OF MULTIDRUG RESISTANCE. Ayala, P; Samuelson, J., Wirth, D. and Orozco, E. Department of Genetics and Molecular Biology, CINESTAV-IPN. Apartado Postal 14-740 Mexico 07000, D.F.; Harvard School of Public Health, 665 Huntington Ave, Boston, MA 02115.

In previous work, mutants of E. histolytica originally isolated by selection in emetine, a commonly used drug for amoebiasis, were shown to have increased resistance to colchicine, an unrelated compound. This phenomenon of cross-resistance to unrelated drugs has been previously observed in multidrug resistant carcinoma cells and the goal of this work was to determine if a similar mechanism existed in E. histolytica. One resistant clone, C2, and the parent clone, A, were analyzed for resistance to other drugs and for the effect of verapamil, a drug known to reverse resistance in multidrug resistant mammalian tumor cells, on resistance to emetine and colchicine. Both C2 and A exhibited similar resistance to both daunomycin (LD50=30uM) and actinomycin D(LD50=13uM). In the presence of verapamil, the LD50 for emetine was reduced from 90 uM to less than 1.8 uM for the C2 clone, while the LD50 for colchicine was reduced from 1.5 uM to 0.5 uM. These results demonstrate that verapamil reverses both emetine and colchicine resistance in the mutant C2 and that the mutant C2 does not show cross resistance to either daunomycin or actinomycin D. In preliminary experiments with 3H-colchicine, accumulation of the drug was reduced in resistant cells. These results are consistent with observations made using multidrug resistant tumor cells and suggest that a P-glycoprotein like molecule may play a role in drug resistance in E. histolytica.

O 503 PURINE TRANSPORT IN GIARDIA LAMBLIA, Kenneth F. Baum, Randolph L. Berens and J. Joseph Marr, Departments of Medicine and Biochemistry, University of Colorado Health Sciences Center, Denver, CO 80262.

The protozoan parasite, Giardia lamblia, is incapable of de novo purine synthesis and must rely on salvage from the environment. This study investigated the first step in this process, nucleoside and nucleobase transport, utilizing rapid uptake determinations. Adenosine and guanosine transport curves appeared to exhibit the hyperbolic kinetics of a saturable carrier-mediated system but at the lowest concentrations of substrate the intra- and extracellular volumes were equal by the time the first measurement was made; therefore, the calculated Km's for transport of 34 µM and 68 µM respectively are probably overestimates. Deoxynucleosides exhibited a much lower affinity for the transporter (Km = 697 µM for deoxyadenosine and 236 µM for deoxyguanosine) in technically more accurate studies. Inhibition studies showed that these purine ribonucleosides and deoxyribonucleosides utilized the same transporter and confirmed their relative affinities for the carrier. The nucleobases, adenine and guanine, did not exhibit saturation kinetics at concentrations up to 1-2 mM substrate. The nucleobases and nucleosides apparently utilized separate carriers, since there was no inhibition of nucleoside transport by nucleobases or vice versa.

Equilibrium exchange studies showed that the carrier is bidirectional with equal velocities in the loaded and unloaded state. When cells were depleted of ATP by 5 mM iodoacetate, the velocity of transport was unchanged. These studies indicate that transport is a passive, saturable process in giardia.

O 504 'TRANSMUTING' HELMINTH ENERGY METABOLISM. Carolyn A. Behm, Eva-M. Bennet, Maureen J. Hanisch and Christopher Bryant, Department of Biochemistry, Faculty of Science, Australian National University, Canberra 2601, Australia.

The energy metabolism of adult *Hymenolepis diminuta* in rats is affected by the immunological or physiological state of the host. 'Normal' parasites produce as metabolic end products succinic, lactic and acetic acids, but under certain circumstances the entire population of worms in an individual rat produces predominantly lactic acid, an energetically less favourable end product. This metabolic 'switch' correlates negatively with the number of parasites that have established in the host. Other evidence suggests that the immunological status of the host may be responsible for this 'switch'. A similar type of change occurs in adults of the intestinal nematode *Heligmosomoides polygyrus* passaged in DBA II mice. A large proportion of the *in vitro* oxygen consumption in 'normal' parasites of this strain is sensitive to antimycin A, the classical inhibitor of phosphorylation Site 2 of the respiratory chain. When the host is concurrently infected with the malarial parasite *Plasmodium vinckei,* oxygen uptake in *H. polygyrus* becomes insensitive to antimycin A. This indicates an apparent loss of aerobic electron transport and associated oxidative phosphorylation, i.e. an energetically less favourable metabolism. These examples of host influence on parasite energy metabolism are significant not only in the understanding of the host-parasite relationship but also in terms of strategies for chemotherapy.

O 505 CHARACTERIZATION OF A ADENOSINE KINASE DEFICIENT MUTANT OF TRYPANOSOMA GAMBIENSE Randolph L. Berens, Jeffrey W. Bowman and Josephine I, Dept. Med. & Biochem., UCHSC, Denver, CO.80262. In order to understand the relationship between the phosphorylation of inosine and adenosine analogues by T. gambiense, mutants deficient in adenosine kinase (AK) were selected for. This was done by mutagenizing procyclic forms growing in a purine and pyrimidine free medium with 25μM xanthine as the purine source. Clones resistant to pyrazofuran at 5 times the ED_{90} of the wild type (WT) were selected for. These clones were then screened for cross resistance to 7-deazaadenosine (7-Ado) at its ED_{90} (2.5 μM). A clone, $2.5/A_3B_6$, that was 30 times more resistant to 7-Ado than the WT (ED_{90}'s of 75 and 2.5 μM respectively) was isolated and characterized. The $2.5/A_3B_6$ was then remutagenized and second clonal population (100/MMJ) resistant to 100 μM 7-Ado was selected for; its ED_{90} for 7-Ado was 500μM. Specific activities(nmol/hr/mg protein) for AK were 57.9, 2.3, and <0.5 respectively for the WT, $2.5/A_3B_6$ and 100/MMJ. Both clones were resistant to other adenosine analogues (aminopurinol riboside, formycin A and 9-deazaadenosine). They were also found to be resistant to inosine analogues (7-deazainosine, formycin B, 9-deazainosine and allopurinol riboside). Preliminary results indicate no differences in nucleoside transport between these mutants and the WT. These data suggest that inosine analogues are phosphorylated by a nucleoside kinase in T. gambiense rather than a nucleoside phosphotransferase as in leishmania.

O 506 IDENTIFICATION OF A MICROTUBULE DRUG WHICH DIFFERENTIALLY INHIBITS LEISHMANIA BUT NOT MAMMALIAN CELL GROWTH. Marion Man-Ying Chan and Dunne Fong, Bureau of Biological Research and Department of Biological Sciences, Rutgers, The State University of New Jersey, Piscataway, N.J. 08855-1059.
Trifluralin is a commercial herbicide which disrupts cell division in root meristems by preventing tubulin assembly into spindle microtubules. However, trifluralin is ineffective in preventing animal cell division. We found that the growth of mammalian macrophage lines (J774, P388D1 murine and HL-60 human) were not inhibited at 50 μM. Since Leishmania and plant tubulins have 80% similarity in their nucleotide sequences, the effect of trifluralin on Leishmania mexicana promastigotes and amastigotes was determined. At 2.5 μM, trifluralin inhibited promastigote growth by 90%. When amastigote-infected macrophages (J774) were treated with 2.5 μM trifluralin in vitro, infection was reduced by 50% in 3 days. Growth inhibition was probably due to blockage of cell division because trifluralin had no effect on the metabolism of non-dividing extracellular amastigotes by viability test. Trifluralin also inhibited the transformation of amastigotes to promastigotes. No promastigotes were detected when amastigotes were cultured at 2.5 μM at room temperature for 3 days, whereas the untreated control consisted mainly of promastigotes. This study shows that trifluralin, which is non-toxic to humans, is potentially an effective drug for treating Leishmania mexicana infection.
This work was supported by the NIH Grant R23 AI-21364 to D.F. M.M.C. is a Busch postdoctoral fellow.

O 507 EXPRESSION OF MALARIA ANTIGENS BY PSEUDORABIES VIRUS, Christina H. Chiang, Scott E. Ashbaugh, Richard D. Macdonald, and Mark D. Cochran, Syntro Corporation, 10655 Sorrento Valley Road, San Diego, CA 92121. The use of live recombinant Pseudorabies Virus (PRV) as a malaria vaccine to protect mice against challenge with Plasmodium berghei and Plasmodium yoelii was investigated. We have constructed a number of PRV recombinants containing the circumsporozoite CS genes from P. berghei and P. yoelii under the control of the PRV glycoprotein (gp) X promoter. The foreign genes were inserted into PRV which have been attenuated for mice. The CS genes were expressed as coding region fusions to various portions of the PRV gpX and gpIII genes. We have determined the level of expression for these recombinants in vitro as well as their ability to sero-convert mice to the CS antigen. The various constructs display varying degrees of in vitro expression which correlate well with their ability to sero-convert mice.

O 508 HIGH RESOLUTION MAPPING OF ANTIGENIC DETERMINANTS ON TRYPANOSOME VARIANT SURFACE GLYCOPROTEIN EXPRESSED IN E. COLI, Vern B. Carruthers, J. Terry Moore and Michael W. Clarke, Department of Microbiology and Immunology, University of Western Ontario, London, ON, Canada N6A 5C1. Variant surface glycoproteins (VSG's) of African trypanosomes pose a structure/function problem that is, in some respects, similar to that of the immunoglobulins. VSG function is, apparently, to present itself on the parasite membrane as the sole antigen inducing neutralizing antibodies. Thus, the role of the VSG is to evoke and bind to serum antibodies. VSG structure must, therefore, be sufficiently variable, at least at sequence level, to generate a large repertoire of serologically distinct variants which appear during antigenic variation. And yet, post-translational processing, intracellular transport and packing require tha all VSG's retain some degree of conserved structure. We have produced non-neutralizing antibodies which bind to linear epitopes present in an isoVAT of four serologically related VSG's. These antibodies are probes of conserved sequence. The determinants bound by these antibodies have been mapped on chemically and enzymatically digested VSG as well as on truncated VSG sequences generated by Exo III digestion of VSG cDNA and expressed in E. coli. The availability of low and high resolution VSG deletion "libraries" enables the rapid sequencing of epitopes bound by anti-peptide antibodies. The presence of such determinants on VSG's outside the isoVAT may indicate conserved sequences required for processing and assembly.

O 509 **BLOODSTREAM TRYPANOSOMA BRUCEI BRUCEI RESPIRATION IS SIMILAR TO PLANT ALTERNATIVE OXIDASE.** **A. B. Clarkson, Jr, G. Poliakis, E. J. Bienen and R. W. Grady.** Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY 10010 and Department of Pediatric Hematology, Cornell University Medical College, New York, NY 10021

The cyanide-insensitive, non-cytochrome-mediated respiratory system of bloodstream African trypanosomes is known to be composed of at least two enzymes, an FAD-containing sn-glycerol-3-phosphate dehydrogenase and an uncharacterized oxidase. A role for CoQ in linking these components has been suggested but never demonstrated. New data clearly show that CoQ_9 does provide this link and furthermore that this system is similar to the alternative oxidase of plants.

The parasites contain 206 ng CoQ_9/mg protein which co-sediments with respiratory actitivty indicating a mitochondrial location. The redox state of this CoQ_9 responds in a manner consistent with function in respiration. The addition of CoQ to acetone extracted cells partially restores respiratory activity. Membrane disruption by digitonin separates the electron transport components but CoQ-containing liposomes recouple the system. A less hydrophobic analoge of CoQ_9-H_2, decyl CoQ-H_2, serves as a direct substrate for the trypanosome oxidase. The non-reduced form of this synthetic substrate recouples digitonin-separated components of the electron transport chain.

Similarities of the trypanosome system to the plant alternative oxidase include: mitochondrial location; CoQ linkage of dehydrogenase and oxidase components; lack of oxidative phosphorylation; and sensitivity to a broad spectrum of inhibitors which selectively block transfer of electrons from CoQ-H_2 to the terminal oxidase but do not block electron transfer to the bc_1 complex of the mammalian cytochrome chain.

O 510 LEISHMANIAL PROTEINASES AS TARGETS FOR DRUG ATTACK, Graham H. Coombs, Christopher A. Hunter and Barbara C. Lockwood, Department of Zoology, University of Glasgow, Glasgow G12 8QQ, Scotland, UK Amastigotes of Leishmania mexicana mexicana contain very high proteolytic activity due to the presence of a group of proteinases of the cysteine type that are located within unusual lysosome-like organelles known as megasomes. Similar enzymes have been detected in stationary phase (infective) populations of promastigotes, whereas cells from log phase do not contain either the enzymes or the organelles. The precise role of the enzymes in parasite survival in macrophages is yet to be elucidated, nevertheless their existence offers possibilities for chemotherapeutic attack of the parasite. Inhibitors of the enzymes, such as antipain, interfere with the growth of the amastigote in macrophages. Another approach is to design prodrugs to be activated by the enzymes. We have shown that antileishmanial amino acid esters such as leucine methyl ester are hydrolysed by the amastigote-specific cysteine proteinases and that the compounds' potent antileishmanial activity is correlated with the presence of megasomes and the cysteine proteinases. It appears that the amino acid esters are acting as prodrugs with the amino acid released upon hydrolysis accumulating in the megasomes with resultant lysis of the amastigote. Currently the enzymes are being characterised in more detail with the aim of designing more specific and potent prodrugs or inhibitors that will be useful antileishmanial agents.

O 511 ANTIBODY PREVENTS ADHERENCE OF TRICHOMONADS TO BOVINE VAGINAL EPITHELIAL CELLS. LB Corbeil[1], JL Hodgson[2], DW Jones[2] and LR Stephens. Department of Pathology, UCSD Medical Center, San Diego, CA 92103[1], College of Veterinary Medicine, Washington State University, Pullman, WA 99164, Res Vet Lab Bairnsdale, Vic., Australia Tritrichomonas foetus colonizes the bovine genital mucosal surface resulting in infertility or an asymptomatic carrier state. Adherence to epithelial cells may be involved in virulence and antibody to trichomonad surface antigens may prevent adherence. To investigate these host-parasite interactions, T. foetus cells were incubated with fresh bovine vaginal epithelial cells (VEC). Trichomonads adhered to squamous VEC at a significantly greater rate than to less differentiated round VEC. Bovine antiserum was shown to react with T. foetus surface antigens by agglutination, immobilization and live cell immunofluorescence. At subagglutinating subimmobilizing dilutions of antiserum, inhibition of adherence of T. foetus to VEC was demonstrated. This antiserum recognized several medium to high molecular weight membrane antigens by Western blotting. Serum fractions enriched in IgG1 inhibited adherence whereas serum fractions enriched in IgG2 did not. The results indicate that antibodies of appropriate isotype and surface specificity may prevent colonization of the vaginal epithelium by T. foetus.

O 512 CONSTRUCTION OF A PLASMID FOR THE PRODUCTION OF SCHISTOSOMAL HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE (HGPRTASE) IN BACTERIA, Sydney P. Craig III, Ling Yuan, James H. McKerrow, John Ademola, and Ching C. Wang, Departments of Pharmaceutical Chemistry & Pathology, University of California, San Francisco, CA 94143

The HGPRTase of *Schistosoma mansoni* plays a pivotal role for survival of the parasite in the human host. HGPRTase catalyzes the salvage of purine bases essential for the synthesis of DNA and RNA. Analysis of the amino acid sequence for this enzyme, deduced indirectly from DNA encoding the HGPRTase (Craig *et al.*, 1988, *Nuc. Acids Res.* 16, 7087-7101) reveal limited differences between amino acids found in the putative active sites for the enzymes of humans and parasites.

In effort to examine the importance of specific amino acids for catalytic function we have constructed an expression plasmid for production of the enzyme in bacteria. In this effort we have ligated cDNA encoding the schistosomal enzyme to DNA with sequences for the bacterial alkaline phosphatase (pho A) promoter and signal peptide (generously provided by Charles Craik of UCSF). The spliced gene was then ligated to a portion of the pBR322 plasmid and the construct, referred to as pexPRT, was cloned in *Escherichia coli*. In theory, the signal peptide for pho (A) should direct the recombinant protein through the plasma membrane into the periplasmic space of the bacteria. Assuming that cleavage of the pho A signal peptide occurs normally, the secreted protein should have only three extra amino acids (isoleucine, arginine, and aspartate) intervening between the arginine that would normally form the amino terminus of phosphatase A, and the methionine that we have previously proposed to represent a probable start codon for the schistosomal HGPRTase.

Preliminary results show that enzymatically active protein is synthesized and is secreted into the periplasmic space of the bacteria transformed with pexPRT.

O 513 USE OF RECOMBINANT ANTIGENS IN ELICITING PROTECTIVE IMMUNE RESPONSE AGAINST TWO SPECIES OF AVIAN COCCIDIA, Harry D. Danforth, Patricia C. Augustine, Robert L. Strausberg, Domenic A. Strolein, Robert A. Clare, and Mark C. Jenkins, U. S. Department of Agriculture, Agriculture Research Service, Livestock and Poultry Science Institute, Beltsville, MD 20705, Genex Corporation, Gaithersburg, MD 20877, and SmithKline Beckman Animal Health Products, P. O. Box 1539, L-34, King of Prussia, PA 19406-0939

The protective immunization elicited with 4 recombinent antigens used singly or in combination against a challenge infection of two species of avian coccidia (Eimeria acervulina and E. tenella) was evaluated in outbred and congenic lines of chickens. The antigens were identified by immuno-screening of cDNA libraries isolated from sporulating E. acervulina or E. tenella oocysts with either immune chicken serum or monoclonal antibodies, and were found to be located on the surface and in internal organelles of the parasites. In outbred birds a single subqutaneous injection at 1 day or 4 weeks of age with 2 of these antigens prepared from E. tenella cDNA elicited significant protective immune response against an infection by this parasite at 2-3 weeks post-immunization (PI). With 6.B congenic birds homozygous for B5, an increase in the amount of antigen coupled with a boost was necessary to elicit significant protection. The other two antigens derived from E. acervulina cDNA each elicited significant protection against an oral challenge infection by this parasite in outbred birds.

O 514 Synthetic Antisense Oligonucleotides for Trypanosome Chemotherapy.

Kim Dix, A. Dave Adams, Andre Lagrange, Charles R. Petrie, John K. Scholler, Diana Sheiness, and Dennis Schwartz. MicroProbe Corporation, Bothell Washington.

All trypanosomal mRNAs appear to possess a unique 5' terminal sequence called the spliced leader. This sequence is an ideal target for antisense oligonucleotide chemotherapy. Antisense oligonucleotides targeting regions within the spliced leader were synthesized with various chemical modifications. 5'-Acridine or 5'-cholesterol conjugated phosphodiester oligonucleotides or methylphosphonate oligonucleotides were employed to enhance either strength of hybridization to target, cell penetration or resistance to nucleases. The modified antisense oligonucleotides were initially characterized by melting temperature (T_M), resistance to nucleases, and the ability to inhibit translation of mRNA in a cell free system. The oligonucleotides then were tested for their ability to kill *T. brucei* by adding oligonucleotides to procyclic cultures and microscopically monitoring cell populations. The results indicate that phosphodiester oligonucleotides containing a 5'-acridine or 5'-cholesterol and methylphosphonate oligonucleotides killed 50% of the cells at 50 and 2 micromolar, respectively. These results have spurred continued research into new modifications of antisense oligonucleotides which will enhance the therapeutic effect in cultured procyclic trypansomes, cultured bloodstream trypanosomes, and, ultimately, trypanosomes *in vivo*. It is anticipated that this research will lead to the development of new class of chemotherapeutic agents that is specific for trypanosomes at low doses with no toxicity to the mammalian host.

O 515 CORRELATION OF RIBOSOMAL RNA STRUCTURE WITH SENSITIVITY TO PROTEIN SYNTHESIS INHIBITORS: GIARDIA AS MODEL, Thomas D. Edlind, Department of Microbiology and Immunology, Medical College of Pennsylvania, Philadelphia, PA 19129

The limited development of anti-parasitic agents targeting protein synthesis stems in part from the belief that parasite and host ribosomes are sufficiently similar to preclude selective toxicity. However, recent studies have revealed that Giardia rRNA has an unusual size and sequence (Edlind & Chakraborty, Nucl. Acids Res. 15,7889-7901). These studies, along with the lack of mitochondria which complicate interpretation of drug sensitivities in other parasites, make Giardia a useful model for the characterization and development of protein synthesis inhibitors as anti-parasitic agents. Three major groups of antibiotics have been evaluated for activity against Giardia using an in vitro growth inhibition assay, and the results have been correlated with sequence and secondary structure analysis of the large (L) and small (S) subunit rRNA: (1) 11 of 13 aminoglycosides tested had little or no activity; the exceptions were hygromycin which targets the universally conserved U-1495 of S RNA and the clinically important paromomycin which targets an adjacent base pair variably present in eukaryotes (Edlind, Antimicrob. Agents Chemother., in press). (2) Of 10 agents tested that target the peptidyl transferase region of L RNA, only anisomycin had significant activity; sequence analysis of this region was completely consistent with these results. (3) The protein synthesis inhibitor most widely used for parasitic infections is tetracycline; while moderately active against Giardia, the more lipophilic minocycline and doxycycline were 10-fold more inhibitory. Studies are in progress to clarify the molecular basis (protein synthesis or membrane transport) for this enhanced activity.

O 516 CLONING AND EXPRESSION OF THE THYMIDYLATE SYNTHASE GENE FROM PNEUMOCYSTIS CARINII, Ursula Edman[1], Jeffrey C. Edman[2], Joseph A. Kovacs[3] and Daniel V. Santi[4], [1]Intercampus Program for Molecular Parasitology and Departments of [2,4]Laboratory Medicine, [4]Biochemistry and Biophysics, and [4]Pharmaceutical Chemistry, University of California, San Fransisco, CA 94143, [3]Critical Care Medicine Department, National Institutes of Health, Bethesda, MD 20892*

A fragment of the thymidylate synthase (TS) gene from P. carinii was synthesized in a polymerase chain reaction (PCR) by the use of 256-fold degenerate oligonucleotide primers complementary to sequences within highly conserved domains of known thymidylate synthases. This PCR product was used as a probe to isolate complete cDNA and genomic DNA clones from respective libraries. Nucleotide sequence analysis demonstrates that the P. carinii TS gene is interrupted by four small introns. The amino acid sequence inferred from the cDNA predicts a protein of 34.2 kD in molecular weight that is 65% homologous to S. cerevisiae TS. Moreover, TS and dihydrofolate reductase (DHFR) are unlinked as Southern blot analysis of transverse alternating field electrophoresis (TAFE) fractionated chromosomes localizes the TS gene to a different chromosome than DHFR. This lends further support to the taxonomic placement of P. carinii as a member of the fungi as TS and DHFR are encoded on a single polypeptide chain in all protozoa examined so far. Expression of catalytically active TS from P. carinii in E. coli allowed purification of the enzyme which is currently the subject of structure-function studies.

O 517 **KINETIC STUDIES OF ENCYSTATION VESICLES AND LOSS OF ABILITY TO ATTACH DURING ENCYSTATION OF GIARDIA LAMBLIA.** Gaetan M. Faubert*, David S. Reiner and Frances D. Gillin. Dept. of Pathology, U.C.S.D., Medical Center San Diego, CA 92103 (*Inst. of Parasitology, McGill Univ., Montreal, Canada).

Little is known about the differentiation of the motile, attachment-competent G. lamblia trophozoite (GLT) into the immobile, wall-encased cyst which is responsible for transmission. We have induced GLT to encyst in vitro and have now begun to analyze this process. The earliest morphologic change is the appearance of large encystation-specific vesicles (ESV) containing cyst antigens which they transport to the cyst wall (Inf. Immun. 3/89). Our studies of the kinetics of ESV appearance and disappearance show that both the percent of cells with ESV and with vesicular localization of cyst antigens increased from 0 to ~45% during the first 18 to 24 hr. in encystation medium. At the same time, the number of ESV/positive cell increased ~4-fold. In contrast, during the second 24 hours, each of these parameters decreased, while the percent of cyst forms and overall staining for cyst antigens increased. Few ESV remain in mature cysts harvested at 42 to 48 hr. In vivo, GLT which remain attached do not encyst. This is reflected in our findings that the relative ability of attached GLT (harvested by chilling at different times) to re-attach decreases from 100% at 18 hr. to <20% after 48 hours in encystation medium. These studies have revealed key physiologic changes in encystation and support the idea that ESV transport cyst antigens to the nascent wall.

O 519 THE MULTIDRUG RESISTANCE GENE (pfmdr) IS AMPLIFIED IN SOME CHLOROQUINE RESISTANT ISOLATES OF **PLASMODIUM FALCIPARUM**, Simon J. Foote, Jennifer K. Thompson, Alan F. Cowman and David J. Kemp, The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia. The multidrug resistant gene from **P.falciparum** (pfmdr) has been isolated and sequenced. It encodes a polypeptide that, like its mammalian homologue (**P.glycoprotein**), comprises an internal duplication, each half having six hydrophobic transmembrane regions plus a hydrophilic region with two nucleotide-binding consensus sequences. Pfmdr is amplified in two chloroquine-resistant isolates. Pulse field gel electrophoretic fractionation of chromosomes places pfmdr on chromosome 5. Macro-restriction mapping of this chromosome has shown that the mechanism of amplification of this chromosome in a resistant South American isolate is quite different to that of an Asian isolate and results in a substantial increase in size of this chromosome. We conclude the presence of an amplified mdr gene in these resistant parasites greatly strengthens the argument that the mdr gene is responsible for chloroquine resistance.

O 520 **CLONING OF A VACCINE SPECIFIC S. MANSONI ANTIGENS AND THE IDENTIFICATION OF A PARTIALLY PROTECTIVE ANTIGEN,** P.H. Francis, Dept. Mol. Rheumatology, MRC Clin. Res. Centre, Watford Road, Harrow and Q.D. Bickle, Dept. Med. Helminthology, London School of Hygiene, and Tropical Medicine, Keppel Street, London WC1E 7HT.

S. mansoni infections attenuated by drug treatment on days 1-3 post-infection induce comparable resistance to that obtained in mice by infection with optimally irradiated cercariae. These experiments demonstrate that the early larval stages possess protective antigens. With the aim of cloning vaccine-specific and possibly larval-specific antigens a cDNA library from the larval progenitor, the sporocyst, was constructed in the expression vector λgt11. Vaccine-specific clones were identified as those recognised by serum from rabbits vaccinated with irradiated cercariae (VRabS) and which were not recognised by serum from mice harbouring single sex infections. VRabS confers protection to mice in serum transfer experiments whereas mice harbouring single-sex infections manifest little resistance to challenge and their serum is not passively protective. Clones recognised specifically by the protective vaccine serum were characterised by "Antibody Select" and were found to correspond to schistosomular antigens of 7, 15, 25 and 130 kD; the latter three are also recognised by serum from vaccinated mice. Further investigations have included the determination of stage-specificity, species-specificity and sequencing analysis. In immunisation studies in mice, the recombinant protein encoding the 130kD antigen conferred low but significant protection in two independent experiments. Investigations to enhance the immunogenicity of this antigen are in progress.

O 521 A CALCIUM BINDING PROTEIN IN BRUGIAN MICROFILARIAE IS DEPENDENT ON AGING, Juliet A. Fuhrman and Willy F. Piessens, Dept. of Tropical Public Health, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115
The monoclonal antibody MF1 has been shown to cause clearance of blood-borne microfilariae (mff) in gerbils infected with Brugia malayi. The MF1 antibody recognizes a proteinaceous determinant on two polypeptides of MW 70 and 75 kD. In Western blots, these two bands show a characteristic shift to lower MW under non-reducing conditions. Both the monoclonal antibody and antisera raised to the gel purified antigen bind only to mature mff. Intrauterine and recently shed mff apparently lack the MF1 molecules. Using rabbit antisera which recognize conserved epitopes of the hsp 70 family, a heat shock cognate of approx. 70 kD molecular weight can be demonstrated in mff extracts. This molecule, however, is distinct from the MF1 antigen, as it can be separated from MF1 by ion-exchange chromatography and SDS-PAGE under non-reducing conditions. Prominent ^{45}Ca-binding molecules comigrate with the MF1 antigens in one-dimensional SDS-PAGE (under reducing and non-reducing conditions). These calcium-binding bands are also restricted to mature mff populations. The MF1 antigen thus appears to be a developmentally regulated calcium binding protein(s).

O 522 ANALYSIS OF THE GROWTH INHIBITION OF LEISHMANIA DONOVANI PROMASTIGOTES BY INOSINE ANALOGS USING A NOVEL COMPUTER AIDED MOLECULAR MODELING PROCEDURE. Arup K. Ghose, J. Joseph Marr, Randolph L. Berens, Vellarkad N. Viswanadhan, Roland K. Robins, and Ganapathi R. Revankar. Nucleic Acid Research Institute, Costa Mesa, CA and Departments of Medicine and Biochemistry, University of Colorado Health Sciences Center, Denver, CO.
Inhibition of Leishmania donovani promastigotes by certain inosine analogues has been analyzed using a novel computer-aided molecular modeling procedure. In this study the minimum energy conformation of formycin B, a pyrazolo (4,3-d)pyrimidine, the most active compound in the data set, was considered as the reference. The low energy conformations of the other molecules were superimposed on it so that the matching of three important physicochemical properties, hydrophobicity, molar refractivity, and atomic charge density, were maximum. The biological activities of these compounds were measured in vitro and expressed as an ED90. These activities were compared with the above properties. The important findings of the study were: (i) the placement of the nitrogen atoms in the heterocyclic ring affects the electron distribution in the ring and, in turn, the biological activity; the more closely the distribution resembles that of the pyrazolo(4,3-d)pyrimidine ring the greater the biological activity; (ii) the substitution at the 8-position may decrease activity by preventing the molecule from taking the *anti* conformation, especially if there is a bulky group; (iii) making the sugar ring more hydrophobic may increase the biological activity.

O 523 ADMINISTRATION OF β-GLUCAN FOLLOWING *LEISHMANIA MAJOR* INFECTION SUPPRESSES DISEASE PROGRESSION IN MICE, Rachel Goldman and Charles Jaffe, Department of Membrane Research and Biophysics, The Weizmann Institute of Science, Rehovot, Israel 76100.
In vivo administration of β-glucans from various sources brings about the stimulation of the reticuloendothelial system leading to macrophage formation, mobilization and activation. This induces nonspecific resistance towards neoplasma, and bacterial and viral infections. The objective of the present study was to evaluate the potential of β-glucan, solubilized from *Saccharomyces cerevisiae*, to suppress the progression of lesions caused by *L. major* in genetically susceptible BALB/c mice when administered post challenge. A high molecular weight gel forming glucan was solubilized from glucan particles by KOH treatment (1M, 60°C for 20 min). The solubilized glucan (in saline) was injected 4 times at 4 day intervals either i.v. or i.p. into mice, infected 4 days previously with a virulent strain of *L. major* (Friedlin, 10^6 promastigotes/ footpad). Control groups which received injections of saline instead of glucan showed a continuous increase in footpad thickness and uniformly developed lesions. Injections of 50μg, 200μg and 400μg glucan per mouse by either route led to reduced footpad swelling and lesion values (0.6-0.8mm footpad thickness as compared to 4.5mm in control groups). Both i.v. and i.p. injection of glucan gave essentially similar effects on *L. major* lesion progression, the effect of 50μg glucan was only slightly less potent than higher doses. Thus, glucan has potential for use in the therapeutic mode to suppress development of cutaneous leishmaniasis.

O 524 SURFACE ANTIGENS OF CRYPTOSPORIDIUM SPOROZOITES AND OOCYSTS. J. Gut and J. Leech. The Medical Service. San Francisco General Hospital. San Francisco, CA 94110. Mouse monoclonal antibodies were generated against Cryptosporidium species isolated from humans with AIDS. The antibodies recognized surface antigens of living and fixed sporozoites and oocysts; they agglutinated living oocysts and sporozoites; and they caused shedding of sporozoite surface coats in vitro. On western blots, the anti-sporozoite antibodies recognized protein antigens with Mrs 40,000 (Mab 13C3), 17,000 (Mab 6C5), and 14,000 (Mab 11A5). One anti-sporozoite antibody (Mab 7B3) recognized two proteins with Mrs >200,00 and 38,000. Anti-oocyst antibodies reacted with multiple protein antigens between Mr 150,000 and Mr 40,000.

O 525 PITFALLS FOR RESEARCH PROGRAMMES INVOLVING THE RATIONAL DESIGN OF ANTIPARASITE DRUGS, Winston E. Gutteridge, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, United Kingdom. Rational drug design programmes for antiparasite drugs understandably tend to focus on the selective interaction of potential ligands with the intended macromolecular target and the absence of such effects on the isofunctional mammalian system. However, it must never be forgotten that a compound intended for systemic administration must also in man be readily mobilisable from the site of administration, be metabolically stable, not be irreversibly bound to plasma proteins, distribute in the body to the particular tissues or organs in which the parasite is located and be able to cross the appropriate membrane systems of both host and parasite to gain access to the intended target. Our recent experiences with the design and development of anti-onchocercal antimycin analogues and antimalarial hydroxynaphthoquinones, both series targetted to the parasites' electron transport systems, highlight the necessity for such consideration early on in the design process. They have also provided us with a salutary lesson: at present such problems can only be approached in a somewhat empirical fashion.

O 526 CHARACTERISATION OF THE GENE FOR A THEILERIA ANNULATA SPOROZOITE SURFACE ANTIGEN WHICH BEARS NEUTRALISING EPITOPES. Roger Hall, Andrew Tait, Brian Shiels, Mark Carrington, Duncan Brown and Susanna Williamson. Wellcome Unit of Molecular Parasitology, Department of Veterinary Parasitology, Bearsden Road, Glasgow, G61 1QH, Scotland, U.K. A sporozoite-surface antigen from Theileria annulata has been characterised using a monoclonal antibody. This antibody inhibits in vitro penetration of sporozoites into bovine peripheral blood leucocytes. The epitope identified resides on four polypeptides expressed in 'mature' sporozoites. These have been shown by cell-free translation to be derived from a single larger precursor polypeptide. The gene for this antigen complex has been cloned and expressed in E. coli. Antibodies to the expressed product inhibit in vitro penetration of sporozoites into leucocytes. Southern blot analysis on DNA from parasite clones demonstrates that this is a single copy gene. Northern blots show that this gene is expressed in a stage specific manner. The complete nucleotide sequence of this gene has been elucidated and shows a number of interesting features, including a repetitive epitope, which will be discussed. This research will provide a basis for the identification of those regions of this antigen which are recognised by bovine helper-T and B cells. As a result, it will be possible to adopt a rational approach to the design of a sub-unit vaccine for T. annulata.

O 527 IDENTIFICATION OF VIRUS-LIKE PARTICLES IN EIMERIA STIEDAE, Hilde Revets, Willy Deleersnijder, Dantcaiel Dekegel, Johan Peeters, Elsy Leysen and Raymond Hamers, Vrije Universiteit Brussel, Instituut voor Moleculaire Biologie, Paardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium

When nucleic acid samples purified from sporozoites of Eimeria stiedae were analysed by gel electrophoresis, an ethidium-stainable band with an apparent electrophoretic mobility of 6.0 kilobase was consistently observed. The band was readily degradable upon RNAse treatment and its susceptibility towards ribonucleaseA on a decreasing ionic strength was suggestive of double-stranded RNA. Electron microscopy revealed icosahedral virus-like particles (VLP) with a diameter of 35nm in sporozoite lysates. The VLP were purified by CsCl buoyant density gradient centrifugation. Upon extraction these particles yielded double-stranded RNA molecules of a uniform length of 1.63 μm. The presence of VLP was investigated in different Eimeria strains. All E. stiedae isolates contained the RNA virus whereas the E. intestinalis and E. magna tested isolates were lacking it.

O 528 INHIBITION OF FILARIAL GLUCOSE TRANSPORT AS A POTENTIAL TARGET FOR .....SELECTIVE CHEMOTHERAPY, David.J.Hayes, Biochemical Sciences, The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, U.K. The filarial parasite Dipetalonema viteae selectively transported D-glucose with a Km of 0.2mM. L-glucose did not impair uptake of D-glucose nor was it metabolised. Glucose uptake was investigated in the presence of various compounds. In common with mammalian cells 2-deoxyglucose, mannose and 5-thioglucose were inhibitory. Glucose transport was not affected by 3-O-methylglucose, cytochalasin B, phloretin, phloridizin, maltose and BMPA-DNP (a bis-mannose affinity label). By use of [3H]-cytochalasin B no glucose displacable binding was observed. Thus, clear differences between the glucose uptake systems of the host and parasite exist. Worms were incubated in glucose-free medium since no inhibitor of filarial glucose transport had been identified. There was a gradual loss in motility and glycogen was depleted by 24 hr. Viability was not maintained under such conditions since 90% of [14C]-adenine previously incorparated into the worms was lost during an in vitro screen. If worms were incubated >18 hr in glucose-free medium no survival was noted on reimplantation into gerbils. Nor did addition of glucose in vitro prevent worm death. Therefore the inhibition of glucose transport may represent a valid target for selective antifilarial chemotherapy.

O 529 MOLECULAR CHARACTERIZATION OF py140 THE SECOND PROTEIN IDENTIFIED ON THE SURFACE OF MALARIA SPOROZOITES. Richard C. Hedstrom, James R. Campbell, Yupin Charoenvit, Mary F. Leef, Martha Sedegah, Ana Szarfman, Walter R. Weiss, Richard L. Beaudoin, and Stephen L. Hoffman. Naval Medical Research Institute, Bethesda, Maryland, 20814.

The circumsporozoite (CS) protein has been the only antigen identified on the surface of malaria sporozoites. In our search for parasite molecules associated with the protection induced by the irradiated sporozoite vaccine, a monoclonal antibody (NYS4) was produced that recognizes a 140 kD protein (py140) found in extracts of Plasmodium yoelii sporozoites. Like NYS1, a monoclonal antibody specific for the CS protein, NYS4 binds to the surface of motile sporozoites and to the material which is shed by live parasites in vitro. In order to define py140, a lambda gt11 expression library was screened with NYS4 and a clone designated M4.2 was selected for DNA hybridization and sequence analysis. M4.2 contains a 2.2kB genomic DNA insert that does not hybridize to the isolated CS protein gene. Furthermore, the genomic organization of the M4.2 fragment is clearly distinguishable from that of the CS protein gene and, in contrast to the latter, shows no restriction fragment length polymorphisms among the DNAs from three P. yoelii parasite clones. The inferred sequence of the M4.2 peptide shows that it does have structural features that resemble those of the CS protein: at least two nonhomologous repeating amino acid sequences and a 20 residue stretch of partial identity with what is referred to as Region II. However, this region is within a larger domain that shares greater homology with a class of mammalian adhesion proteins. Thus, the sporozoite surface of P. yoelii is composed of at least two structurally similar proteins both of which are candidates for vaccine development.

O 530 PHOSPHORYL-CHOLINE AND NON PHOSPHORYL-CHOLINE CONTAINING ANTIGENS IN TRICHINELLA SPIRALIS, Wieger, L.Homan, Anja C.G. Derksen and Frans van Knapen, Department of Parasitology and Mycology.
National Institute of Public Health and Environmental Protection, P.O.Box 1, 3720 BA Bilthoven, The Netherlands.
Several antigens were identified that elicit an antibody respons in animals infected with the nematode T.spiralis. Among them phosphoryl-choline (PC) bearing components were the first to be recognized in the cause of an infection. Antibodies to these components were directed to phosphoryl choline and crossreacted via this hapten with antigens from several other parasites. Parasite-specific epitopes present on PC bearing components could be identified however. A monoclonal antibody to such an epitope is prepared for use in protection studies. Among the non-PC bearing components a protection inducing glycoprotein is identified. A monoclonal antibodie to this T.spiralis specific secreted antigen recognize a repetitive epitope, that is also detectable on the surface of muscle larvae. The isolation and characterization of these antigens are described.

O 531 THREE DIMENSIONAL STRUCTURE OF THE GPI ANCHOR GLYCAN OF T. brucei, Steven W. Homans, Christopher J. Edge, Michael A.J. Ferguson, Raymond A. Dwek and Thomas W. Rademacher, Department of Biochemistry, University of Oxford OX1 3QU, U.K. and Department of Biochemistry, University of Dundee DD1 4HN, U.K.
The parasitic protozoan Trypanosoma brucei undergoes a complex life-cycle between an insect (tsetse fly) vector and its mammalian hosts. T. brucei is the causative agent of ngana in cattle and is closely related to T. rhodesiense and T. gambiense which cause human sleeping sickness. The parasite lives in the blood and lymph of the mammalian host where it is protected from lytic serum components by a dense monolayer of variant surface glycoprotein (VSG) which forms a continuous macromolecular diffusion barrier. The VSGs of all African trypanosomes are anchored to the plasma membrane via covalent linkage of the COOH terminal amino acids to glycosyl-phosphatidylinositol (GPI) moieties, rather than via transmembrane hydrophobic polypeptide sequences. Using a combination of two-dimensional 1H-1H NMR methods together with molecular orbital calculations and restrained molecular dynamics simulations, the average solution conformation of the GPI membrane anchor glycan of T. brucei VSG has been determined. This shows that the glycan exists in an extended configuration along the plane of the membrane and spans an area of 600A^2, which is similar to the cross-sectional area of a monomeric N-terminal VSG domain. Taken together, these observations suggest a possible space-filling role for the GPI anchor which may maintain the integrity of the VSG coat. The potential importance of the GPI glycan as a chemotherapeutic target will be described in the light of these observations.

O 532 *PLASMODIUM FALCIPARUM* RHOPTRY PROTEINS. Randall F. Howard, Oded Arad, and Robert T. Reese, Agouron Institute, 505 Coast Blvd. S., La Jolla, CA 92037.
The rhoptries of the asexual erythrocytic stage of *P. falciparum* have been suggested to be involved in the lysis and/or invasion of the erythrocyte by the parasite. Our rhoptry-specific monoclonal antibodies immunoprecipitate proteins of 82 kDa, 70 kDa, 67 kDa, 39 kDa, and 37 kDa (SDS-polyacrylamide gel electrophoresis under reducing conditions). The proteins p39 and p37, however, lack epitopes for these monoclonal antibodies and probably bind directly or indirectly to p82, p70, or p67 as shown by immunoblot analyses and detergent dissociation experiments. Immune complexes containing p39 and p37 lacked aldolase activity and therefore probably are not related to the M_r 41k protein identified by Certa et al. ([88] *Science* **240**:1036) as being a rhoptry-associated antigen which has sequence homology to class I aldolases and exhibits aldolase activity. We have synthesized two peptides that model regions on the surface of *P. falciparum* aldolase (PfA). The choice of the sequences was based on the assumption that the PfA has a structure similar to rabbit muscle aldolase (RMA), a class I enzyme whose 3-D structure has been determined to 2.7 Å (Sygusch et al. [87] *Proc. Natl. Acad. Sci.* **84**:7846). Peptide IV-9 (residues 117-133 of PfA) modeled what resembles a β turn - random coil region in RMA, and peptide IV-10 (residues 311-325) modeled a β turn - α helical region. In these two regions, there are 5 and 6 amino acid substitutions (all but one nonconservative), respectively, in PfA relative to RMA. These peptides were conjugated to KLH and are being used to raise mouse and rabbit anti-PfA antibodies. Experimental results with these antisera will be reported.

O 533 **DEFINED PROTEINS FROM LEISHMANIA DONOVANI FOR SERODIAGNOSIS AND VACCINATION**, Charles L. Jaffe, Rive Sarfstein, Nurit Rachamin and Mariano Zalis, Dept. of Biophysics-MacArthur Center for Molecular Parasitology, Weizmann Institute of Science, Rehovot, Israel 76100.
Development of good diagnostic assays and effective vaccines for the fatal disease, visceral leishmaniasis (VL), is of utmost importance. Two L.donovani proteins recognized by VL patient sera, 70 and 72kDa, were purified by affinity chromatography and HPLC. These proteins were shown to be different by peptide mapping and western blotting. In a direct dot-ELISA using gp70-2, 90% (36/40) of the VL sera were identified; 1.2% (1/86) of the non-VL sera were misdiagnosed. Similar results were obtained with dp72, 100% (21/21) of the VL sera were diagnosed; 7% (5/71) of the non-VL sera were incorrectly identified. Both proteins exhibit microheterogenity on SDS-PAGE, futhermore the M_r of the dp72 was shown to vary between isolates of L.donovani. Analysis of additional leishmanial species identified protein(s) of lower M_r which reacted with D13 (anti-72kDa), but not with D2 (anti-70kDa). Both proteins appear to be glycosylated and are present in promastigotes, as well as amastigotes. Mice (Balb/c) were immunized with the 70 and/or 72kDa proteins (12 μg) from L.donovani and challenged, intravenously, with 5 x 10^6 L.donovani amastigotes. A reduction in the parasitemia of greater than 70% was observed in those mice which received the 72kDa protein. Work is in progress to elucidate the mechanism of the observed protection.

O 534 Altered Expression of Muscle Proteins in Trichinosis. Douglas P. Jasmer, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164. Infection by Trichinella spiralis larvae causes significant alterations in mammalian muscle morphologically, ultrastructurally and biochemically. To characterize the phenotype of the infected cell and identify the level at which these alterations are regulated, muscle specific gene products were compared between skeletal muscle and the infected cell. A new method to isolate T. spiralis infected cells in preparative quantities was developed. When compared to muscle by SDS-PAGE, the relative abundance of most major muscle proteins was reduced in infected cells. Using a monoclonal antibody to the heavy chain of skeletal muscle myosin indicated a minimum 100 fold reduction of this protein in nurse cells. Muscle specific alpha-actin was undetectable in infected cells by 2-D PAGE analysis. In contrast, numerous proteins associated with the organellar fractions of the the infected cell were not detected in muscle by the methods employed. These observations suggest either that the infected cells is not a regenerating muscle cell, or that their is a block in the normal regeneration process of the infected muscle cell. RNA transcripts for several muscle specific genes are currently being analysed to help distinguish between these possibilities.

O 535 *ACANTHOCHEILONEMA VITEAE*: CHARACTERIZATION OF ES-ANTIGENS OF INFECTIVE LARVAE BY MONOCLONAL ANTIBODIES, Brigitte Kaltmann*, Shailja Misra*, Werner Rudin** and Richard Lucius*, *Institut für Tropenhygiene, Im Neuenheimer Feld 324, 69 Heidelberg, FRG. ** Schweizerisches Tropeninstitut, Socinstr. 57, 4051 Basel, Switzerland.
Infective larvae (L3) of *A.viteae* obtained from ticks synthetize various antigens as shown by metabolic labelling and export them into culture supernatant (CSN). CSN repeatedly adminstered with alum induced a resistance of 51% against challenge infection in jirds and was analysed with mAbs in order to identify potentially protective antigens. The most prominent antigen is shed in soluble form during the first 48 hrs of culture. It is frequently recognized by sera of infected jirds which are partially resistant against challenge infection. In L3 a target epitope is present in the esophagial glands in EM-sections and on a triplet of bands in immunblots. In adult female worms the mAb recognizes the surface of mature uterine microfilariae, but not of blood microfilariae. The target antigen is shed into culture as particles. It is only soluble in SDS-2ME and has a MW of 26 KD. This correlation suggests that female filariae shed an antigen crossreacting with the major immunodominant antigen of L3.

O 536 CHARACTERISATION OF P.YOELII PROTECTIVE ANTIGENS. Keen. J.K.. and Playfair. J.H.L.* Department of Molecular Biology. Wellcome Biotech. Langley Court. Beckenham. Kent. BR3 3BS. *Department of Immunology. University College and Middlesex School of Medicine. Arthur Stanley House. Tottenham Street. London. W1P 9PG. A monoclonal antibody. 25.77. recognising P.yoelii merozoites converts fulminating infections to self limiting infections on passive transfer into infected mice. (Freeman et al. 1980). The monoclonal antibody precipitates a 235.000 MW protein which is protective when used to immunise mice. The protein has been localised to the rhoptries of P.yoelii merozoites. (Oka et al. 1984). Rhoptries release proteins which may facilitate erythrocyte membrane invagination and invasion by the malarial parasite. A lambda gt11 genomic clone expressing 100 base pairs of Eco R1* cut P.yoelii DNA was used to isolate antibodies from a polyvalent hyper-immune serum raised against P.yoelii (Ozaki et al. 1986). Selected antibodies recognise individual late stage merozoites and precipitate a parasite protein of 235.000 MW. The lambda gt11 clone hybridises to multiple bands on Southern blots of RSA I and Dra I digested P.yoelii genomic DNA. The same clone was used to isolate pUC13 recombinants containing inserts of P.yoelii Dra I cut DNA ranging in size from 1-7KB.

O 537 ORNITHINE DECARBOXYLASE AND TRYPANOTHIONE REDUCTASE GENES IN Leishmania braziliensis guyanensis, Janet S. Keithly, Department of Medicine, Cornell University Medical College, New York, NY 10021, and Director of Parasitology, The Wadsworth Center for Laboratories and Research, New York State Department of Health, Empire State Plaza, Albany, NY 12201. In the Order Kinetoplastida ornithine decarboxylase (ODC) and trypanothione reductase (TR) are the key enzymes for polyamine and trypanothione biosynthesis. These metabolic pathways are functionally linked and are essential for kinetoplastid growth, differentiation, and survival. Using a heterologous Trypanosoma brucei brucei probe for ODC, and a mixed synthetic oligonucleotide for TR, we have detected the putative genes for these enzymes by Southern blot hybridization using genomic DNA of Leishmania braziliensis guyanensis MHOM/SR/80/CUMC 1. The TR synthetic probe is a mixed oligomer of 29 nucleotides. It was constructed from the known codon usage for other flavin oxidoreductases over a wide evolutionary scale, and preferred codon usage for species of Leishmania. The results suggest that ODC and TR are single genes in this species of Leishmania, and that these probes will be useful for isolation and characterization of the genes.

O 538 SEQUENCE ANALYSIS AND MOLECULAR MODELLING OF GLYCOSOMAL GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) IN Trypanosoma cruzi, Giles Kendall[1], Andrew F. Wilderspin[2], Frank Ashall[1], Michael A. Miles[1] and John M. Kelly[1], [1]Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK., [2] Laboratory of Molecular Biology, Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, UK.
A T. cruzi genomic DNA library was screened with a glycosomal GAPDH cDNA probe derived from Trypanosoma brucei. A clone was isolated which contained two tandemly repeated genes. Although the inferred amino acid sequence was 90% similar to that of the T. brucei enzyme there was sequence divergence in a region thought to be important for the transport of GAPDH into the glycosome. This change, together with molecular modelling data, has implications for our understanding of the mechanisms involved in the intraglycosomal localisation of the enzyme. The intergenic region of the T. cruzi genes (517 bp) contained a sequence with the properties of a retroposon. It has a polyadenylated segment and is flanked by short direct-repeat sequences. The retroposon-like element also occurs in a truncated form on either side of the gene repeat and is transcribed as part of the untranslated region of the mature GAPDH mRNA.

O 539 THE 14 kDa ANTIGEN OF *ASCARIS* : ALLERGENICITY, MHC-RESTRICTED RECOGNITION, AND AMINO ACID SEQUENCE.

Malcolm W. Kennedy, Lesley A. Tomlinson Jacqueline F. Christie and Eleanor M. Fraser. Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden, Glasgow G61 1QH, Scotland, U.K.

The 14 kDa antigen of the nematode *Ascaris* is a major internal protein of the parasite, and is also released during culture *in vitro* by the tissue-penetrative larvae and adult worms. Humans infected with *Ascaris lumbricoides* vary considerably in antibody responsiveness to this molecule, and this heterogeneity can be modelled in inbred laboratory rodents. In the case of the latter, the response to the 14 kDa is MHC-restricted in both rats and mice. Only mice of H-2^S and rats of $RT1^U$ have, so far, been found to be responders, and this restriction only operates in the context of infection. Assay of the IgE response in these animals by Passive Cutaneous Anaphylaxis showed that the above MHC restriction also applied to the specificity of the reaginic antibody response. Animals of all MHC haplotypes, however, responded to other *Ascaris* allergens. Amino acid analysis of the 14 kDa equates it to a previously identified "Allergen A" of the parasite, and its sequence is now available. These findings have implications for the genetic control of allergic responses in general, and, in particular, to the hypersensitivity responses which are a significant feature of infections with parasitic nematodes. There are also implications for the generation of hypersensitivity responses by recombinant vaccines involving certain parasite components.

O 540 STUDIES ON THE CELLULAR BIOLOGY OF CALCIUM AND MOLECULAR BIOLOGY OF A PUTATIVE CATION-MOTIVE ATPase IN PLASMODIUM.

S. Krishna, L.H. Bannister[1], G.G. Germino, R.A. Wells, M. Whittaker[2], K.J.H. Robson. MRC Unit of Molecular Haematology, Nuffield Dept. Clin. Med., John Radcliffe Hosp., Oxford, OX3 9DU. [1] Dept. Anatomy, Guy's Hosp., London SE1, [2]Dept. Clin. Phys., Univ. Coll. Hosp., London WC1E.

During its erythrocytic cycle, Plasmodium falciparum undergoes structural alterations which reflect complex and poorly understood changes in its biochemistry. We have studied the physiology of Ca^{2+} metabolism in synchronized cultures of P. falciparum and shown that ^{45}Ca uptake increases particularly at schizogony. This uptake is associated with the appearance of a hitherto undescribed class of small (100nm) membranous vesicle, containing dense granular material extractable with EGTA. The numbers and electron density of these vesicles increases markedly at the end of schizogony and in merozoites but decreases during the early stages of post-invasion growth when ^{45}Ca uptake is low. Ca^{2+} uptake into the vesicles in the Ca^{2+} poor environment of the red cell may require an energy dependent concentrating mechanism. Consequently we have proceeded to isolate a 450bp clone coding for a putative cation-motive ATPase from a genomic DNA library, using an oligonucleotide probe derived from amino acid sequences conserved in other ATPases. The derived amino acid sequence from this clone demonstrates some highly conserved motifs present in the K^+-ATPase of E. coli and rabbit fast skeletal Ca^{2+}/Mg^{2+} ATPase. Further studies are being carried out to determine the full structure and function of this interesting sequence and these vesicles.

O 541 REVERSAL OF DRUG RESISTANCE IN Plasmodium falciparum in vitro: IMPLICATIONS FOR DRUG DESIGN.

Dennis E. Kyle, W.K. Milhous and A.M.J. Oduola, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

In mammalian neoplastic cells acquired resistance to one drug may confer resistance to other drugs of the same chemical class as well as resistance to structurally and functionally unrelated compounds. This is apparently without an exact parallel in Plasmodium falciparum where a consistent pattern of cross-resistance to chloroquine and other antimalarials has been hard to establish. Recent studies with resistant P. falciparum suggest at least one analogous mechanism of resistance, with the most striking similarity being the reversal of resistance by many of the same calcium antagonists. We have examined the ability of calcium antagonists to reverse resistance in vitro to new and currently used antimalarial drugs and have identified two "reversal" phenotypes. The first is the reversal of resistance to chloroquine by a variety of calcium antagonists (e.g., verapamil, chlorpromazine, and desipramine). In most of these chloroquine resistant parasites, the same resistance modulators also reverse resistance to the primary metabolite of chloroquine (desethyl-chloroquine) and to a series of chinchona alkaloids (quinine, quinidine, chinchonine, and chinchonidine). In a second group only penfluridol (WR 256473) circumvented resistance (or reduced susceptibility) to the quinoline methanol mefloquine, the phenanthrene methanol halofantrine, and the sesquiterpene lactone artemisinin. These results suggest that the mefloquine resistance phenotype affected by penfluridol may be more akin to "classical" multidrug resistance in mammalian cells and may play a role in future strategies for use of new drugs in areas where this phenotype exists.

O 542 UTILIZATION OF A BIOCHEMICAL APPROACH IN THE DEVELOPMENT OF BENZIMIDAZOLES (BZs) AND RELATED INHIBITORS WITH ACTIVITY AGAINST BZ-RESISTANT NEMATODE ISOLATES, Ernest Lacey, CSIRO Division of Animal Health, McMaster Laboratory, Private Bag No.1 PO Glebe, NSW 2037. Benzimidazole (BZ) anthelmintics act by binding the dimeric soluble protein, tubulin, at the colchicine binding site with resultant inhibition of polymerization to the cytoskeletal microtubule matrix. The use of these compounds against the ruminant parasitic nematodes, Haemonchus contortus, Trichostrongylus colubriformis, Ostertagia circumcinta and Nematodirus spathiger has lead to the development of BZ resistance. This resistance is due to a change in the ability of BZ to bind the tubulin of these species. Investigation of the extent of this change among the commercially available benzimidazoles demonstrated that the resistance factors (RF, ratio of binding to BZ-S and BZ-R tubulin) were lower in the 5-substituted phenyl derivatives, mebendazole (RF=7), oxfendazole (RF=15) and fenbendazole (RF=10) than the 5-substituted alkyl derivatives, albendazole (RF=61), oxibendazole (RF=69) and parbendazole (RF=70). As the interaction of these BZ carbamates (as opposed to the non-carbamate, thiabendazole) is pseudo-irreversible (association rate 3 pmol/min, dissociation rate 0.007 pmol/min), a pre-incubation technique with unlabelled inhibitors followed by incubation with [3H] mebendazole was developed to enable the identification of microtubule inhibitors which showed no specificity between S and R isolates. Resistance factors derived from these experiments, parallelled those obtained in an in vitro development assay (egg to L_3 development), supporting the relationship between the biochemical interaction and pharmacology. By these techniques, several classes of microtubule inhibitors have been identified which exert activity against both BZ-S and R isolates, most notable among these are the deazadihydropteridines. Optimisation of the BZ structure has also lead to the reduction of RFs to generate new benzimidazoles which show good activity against BZ-R isolates.

O 543 **THE 230000 MOLECULAR MASS MEROZOITE SURFACE ANTIGEN OF PLASMODIUM YOELII: CLONING AND ANALYSIS OF THE 3' HALF OF THE GENE.**

Alan P.Lewis, Department of Molecular Biology, Wellcome Biotech, Langley Court,Beckenham, Kent, England, BR3 3BS.

The precursor to the major merozoite surface antigen is a candidate for the development of a vaccine against the asexual blood stage of malaria. This polypeptide has a molecular mass of 230000 in the murine malaria, P.yoelii, and provides a rodent model as an aid to vaccine development. Oligonucleotide probes corresponding to conserved regions of the published 3' portion of the P.yoelii 17XL PY230 gene (Burns, J.M. et al, PNAS, 85:602,1988) were used to probe P.yoelii YM genomic libraries in Lambda gt11 and pUC9 for PY230 sequences. Two recombinant clones were obtained possessing overlapping DNA sequences, which contained the 3' 3.3 kilobases of the PY230 gene. A region of this sequence detected an RNA species of 7.3kb in an RNA Northern blot of total P.yoelii YM RNA. Nucleic acid sequence analysis has revealed that the terminal 2kb of the gene is identical to the previously published region from the 17 XL strain of P.yoelii. The remaining 1.3kb has been compared with the equivalent region in the Plasmodium falciparum PF195, and spans the sequence analogous to that encoding the 28-30 kD PF195 processed product. The PY230 amino acid sequence contains an extra 113 residues arranged predominantly as two stretches situated within regions of approximately 20% amino acid homology. It also possesses two blocks of high homology (60-70%) corresponding to PF195 sequences semi-conserved between P.falciparum strains, within which are two conserved cysteine residues.

O 544 CLONING AND CHARACTERIZATION OF A PROTECTIVE ANTIGEN FROM EIMERIA TENELLA, Paul A. Liberator, Helen Profous-Juchelka, Jennifer L. Weimer, Mark Crane and Mervyn J. Turner, Department of Biochemical Parasitology, Merck, Sharp & Dohme Research Laboratories, Rahway, NJ 07065-0900

Clone SO7 was isolated by immunological screening of a cDNA library constructed with poly(A) RNA from Eimeria tenella sporulating oocysts using anti-E. tenella sporozoite antibody. Clone SO7 detects a single species of mRNA 1.2kb in length on a Northern blot, which is present prior to and during sporulation. Western blot analysis using mono-specific recombinant eluted antisera highlights a group of E. tenella sporozoite proteins in the 27-28kD range, whose synthesis is coincident with the onset of sporulation. Deduced amino acid sequence from the nucleotide sequence of clone SO7 and overlapping cDNA clones shares no significant homology with anything in the database. A DNA repetitive element is located within the 3'untranslated region of the cDNA. Genomic clones have been isolated and together with genomic Southern blots, indicate that SO7 is encoded for by a single copy gene with no large intervening sequences. Primer extension and S1 nuclease analysis indicate that our longest cDNA is 17nt from the 5'end of the mRNA and that those 17nt are coincident with the genomic sequence. Nucleotide sequence upstream from the putative start of transcription bears no homology to RNA polymerase II consensus sequences. When expressed as a recombinant bacterial fusion protein, this antigen is capable of protecting chickens from subsequent challenge with live E. tenella oocysts.

O 545 PURIFICATION AND CHARACTERIZATION OF NUCLEOSIDE CLEAVING ACTIVITIES IN TRYPANSOMA GAMBIENSE S. A. Long-Krug, Josephine I and R. L. Berens. University of Colorado Health Sciences Center, Denver, Co. 80262. In parasitic hemoflagellates, the major route of purine nucleoside salvage is through cleavage of nucleotides to their respective bases. Three separate nucleoside cleavage activities were found in Trypanosoma gambiense by the following procedure. Affinity FPLC of crude extracts of T. gambiense procyclic forms was conducted by placing them over an ^{6}N AMP sepharose column and washing with 50 mM PIPES (pH 6.8) containing 1 mM DTT and 10% glycerol. Phosphorylytic activity which metabolized adenosine was bound and was eluted with 5 mM adenosine or ATP in Tris (pH 8.8) containing 1 mM DTT, 10% glycerol and 500mM KCl. Unbound protein containing nucleoside cleavage activities was concentrated by precipitation with 95% ammonium sulfate. The precipitate was solubilized in 50mM PIPES and applied to a Superose 6 FPLC sizing gel. Two hydrolytic activities were eluted with 50 mM PIPES containing 200 mM KCl. The first had a molecular weight of ≈158,000 and metabolized the ribonucleosides inosine, guanosine, adenosine and xanthosin. The second had a molecular weight of ≈17,000 and metabolized the deoxyribonucleosides deoxyinosine, deoxyguanosine and deoxyadenosine. Kinetic parameters of these enzymes will be presented.

O 546 THERAPEUTIC EFFICACY OF ALLOPURINOL IN PATIENTS WITH CHRONIC CHAGAS' DISEASE. Rafael H Gallerano, Raul R Sosa, and J Joseph Marr, Department of Medicine,Medical School of the National University of Cordoba, Cordoba, Argentina and Departments of Medicine and Biochemistry,University of Colorado Health Sciences Center, Denver, CO.
Laboratory and animal studies have demonstrated that purine analogues, especially pyrazolopyrimidines, have significant activity against Trypanosoma cruzi. A four year clinical investigation was undertaken to compare the efficacy of allopurinol (4-hydroxy(3,4-d)pyrazolopyrimidine) with current nitrofuran therapy in the treatment of chronic Chagas' Disease. Three hundred and seven patients were studied; 91 were untreated and the remaining 216 were divided into four treatment groups: allopurinol, 600 or 900 mg/d; benznidazole,5 mg/kg/d; or nifurtimox, 8-10 mg/kg/d. All drugs were given for 60 days. Patients were evaluated clinically, serologically, and parasitologically. Allopurinol is as efficacious as the nitrofurans in eliminating the parasitemia and rendering patients seronegative. Adverse reactions occurred in 11% of patients who received allopurinol and in 30% of those receiving the two nitrofurans. The reactions to the conventional therapy not only were more frequent but were of a more serious nature. Allopurinol is an inexpensive, nontoxic, oral agent which is efficacious for the treatment of chronic Chagas' Disease.

O 547 ACTIVATED OXYGEN MEDIATES THE ANTIMALARIAL ACTIVITY OF QINGHAOSU, S.R. Meshnick, Lin, F.B., Pan, H.Z., Chang, C.N.,Kuypers, F., Chiu, D., and Lubin, B.; City University of New York Medical School, New York, NY 10031; Peking Union Medical College, Beijing, China; Childrens Hospital Oakland Research Institute, Oakland CA 94609.
Qinghaosu (artemisinin), is an important new antimalarial which is derived from an ancient Chinese herbal remedy. Because it contains an endoperoxide bridge which is essential for its antimalarial activity, it has been suggested that qinghaosu, like other peroxides, kill malarial parasites by generating activated forms of oxygen. We have obtained the following pieces of evidence consistent with the proposal that activated oxygen mediates the antimalarial activity of qinghaosu: 1) Artesunate, a water-soluble derivative of qinghaosu, spontaneously generates activated forms of oxygen at elevated pH's; 2) Artesunate stimulates the generation of thiobarbituric acid reacting substances (TBARS) in red cells. This generation is faster in infected red cells than in uninfected red cells; 3) In isolated red cell membranes, qinghaosu initiates lipid peroxidation, and the production of lipid peroxides. Both processes are dependent on the presence of metals, and do not occur with deoxyqinghaosu (an inactive congener which contains an epoxide instead of an endoperoxide); 4) Ascorbic acid and reduced glutathione (GSH), two antioxidants, inhibit the antimalarial activity of qinghaosu in vitro.

Drug and Vaccine Design; Pathogenesis and Virulence-II

O 548 TUMOR NECROSIS FACTOR (TNF) AND THE ANEMIA OF MALARIA, Kathleen L. Miller, Paul H. Silverman and Birgitta Kullgren. Division of Cell and Molecular Biology, Lawrence Berkeley Labs, University of California at Berkeley, Berkeley, CA. 94720

The anemia associated with malaria is complex and multiple factors contribute to its severity. An increased destruction and a decreased production of red blood cells appears to be involved, however, the mechanisms remain unclear. The macrophage cytokine TNF is thought to play a role through its ability to inhibit erythropoiesis. To study the cellular mechanisms involved in the ineffective erythropoiesis, we have examined erythropoiesis in mice infected with Plasmodium berghei and in mice infused with recombinant TNF, via implanted osmotic pumps. In both groups of mice there were; 1.) a reduction of pluripotent stem cells (CFU-S) in the bone marrow and an increase in the spleen, 2.) a reduction of erythroid progenitor cells (CFU-E and BFU-E) and 3.) a reduced incorporation of ^{59}Fe into red blood cells. When P. berghei infected mice were given antisera against recombinant murine TNF, erythropoiesis was partially restored. These results demonstrate that TNF mediates, in part, the anemia associated with malaria. How TNF may act, directly or indirectly, to inhibit erythropoiesis is not yet clear.

O 549 LYMPHOKINE PRODUCTION BY MURINE SPLEEN CELLS DURING *TRYPANOSOMA CRUZI* INFECTION. Gary S. Nabors, Gina R. Benavides and Rick L. Tarleton. Department of Zoology, University of Georgia, Athens, GA 30602. Murine infection with *Trypanosoma cruzi* results in a severe and prolonged suppression of both humoral and cellular immune responsiveness including suppression of IL-2 production. In the current study, we have examined the production in *T. cruzi*-infected mice of lymphokines other than IL-2 to determine if suppression of lymphokine production is specific for IL-2, specific for lymphokines produced by a certain subset of T cells, or non-specific for all lymphokines. Spleen cells from infected C57Bl/6 mice which are suppressed with respect to IL-2 production are capable of producing both interferon gamma (IFN-γ) and IL-3 at or above the amount produced by stimulated spleen cells from non-infected mice. IFN-γ activity was detected by using a IFN-γ specific ELISA and by a viral cytopathic effect inhibition assay. The IFN produced by cells from infected animals was not totally abrogated by pH2 treatment, suggesting that both IFN-α/β and IFN-γ are present in these supernatants. The majority of IFN-γ production was abrogated by treatment of spleen cells with anti-CD8 antibody and complement, suggesting that a large percentage of the IFN-γ in this system is the product of CD8+ T cells. In the highly susceptible C3H mouse, stimulated spleen cells suppressed for IL-2 production are also capable of producing a greater amount of IFN-γ than spleen cells from non-infected mice. These data indicate that *in vitro* suppression of lymphokine production during *T. cruzi* infection does not extend to the production of all lymphokines, but may be specific for lymphokines produced by the TH1 population of T cells. However, it is unknown whether *in vitro* lymphokine production by mitogen-stimulated spleen cells is an accurate representation of lymphokine production occurring *in vivo* during an active infection. We are currently using murine lymphokine cDNA probes to detect lymphokine mRNAs in order to examine this question.

O 550 CELLULAR IMMUNE RESPONSES IN HUMAN VOLUNTEERS IMMUNIZED WITH IRRADIATED PLASMODIUM FALCIPARUM SPOROZOITES. Elizabeth H. Nardin, Deidre Herrington, Myron Levine, James Murphy, Jonathan Davis, Victor Nussenzweig and Ruth S. Nussenzweig, Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY 10010 and Center for Vaccine Development, Baltimore, MD 21201.

Immunity to the sporozoite stage of the malaria parasite includes both antibody and cell mediated facets of the immune response. Human volunteers can develop protective immunity after multiple exposures to the bites of irradiated malaria-infected mosquitoes, but the cell mediated immune responses in such sporozoite-immunized volunteers have not been investigated previously. Peripheral blood lymphocytes (PBL) obtained from three seropositive sporozoite immunized volunteers proliferated when challenged in vitro with a recombinant P. falciparum CS (rPfCS) protein which contains approximately 70% of the total CS protein. PBL obtained from the volunteers prior to immunization and PBL of normal individuals did not proliferate in response to challenge with rPfCS. T cell lines and clones were obtained from one volunteer by in vitro expansion of the antigen specific cells using rPfCS and IL-2. A total of 40 CD4+ T cell clones were obtained which gave stimulation indices ranging from 2.5 to 103.4 when challenged with 10 ug/ml of rPfCS. Five CD4+ clones were chosen for further analysis. The clones are specific for P. falciparum CS and do not proliferate when challenged with a recombinant P.vivax CS protein or with a yeast extract control. High levels of gamma interferon (27 - 80 U/ml) were produced by the clones following antigen stimulation in vitro. The fine specificity of the T cell clones is being examined using synthetic peptides constructed to overlap the entire CS sequence of the NF54 strain of P. falciparum.

O 551 THE HUMORAL RESPONSE OF MALARIA PATIENTS TO P. FALCIPARUM GAMETOCYTE SURFACE ANTIGENS (POTENTIAL TRANSMISSION BLOCKING VACCINE CANDIDATES), Corinne S.L. Ong, Patricia M. Graves, Stephen J. Eida, Kun-Yan Zhang, Martin Looker, Neil C. Rogers, Peter Chiodini* and Geoffrey A.T. Targett, Departments of Medical Parasitology and *Clinical Sciences, London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K..

Serum samples from patients in London who have contracted malaria abroad and schoolchildren in Papua New Guinea were studied using a range of immunological techniques. Sera of individuals from hyperendemic areas have been found to immunoprecipitate the 230 and 48/45 kD gametocyte surface antigens which are the targets of transmission blocking antibodies. When tested by immunofluorescence, the patients in this study were found able to mount antibody responses to these antigens even during their first attack of malaria with antibody titres that were comparable to another group of patients who had had two or more previous attacks of malaria. In competitive ELISAs, a third of the sera (at a 1/10 dilution) inhibited the binding of at least two transmission blocking MAbs to different epitopes on the 48/45 kD antigen, one of which is on a linear and non-carbohydrate section of the 45kD glycoprotein. Variable responses were found on Western blotting with some sera recognising the 230 kD as well as the 48/45 kD antigens . These results are promising for the development of malaria transmission blocking vaccines.

O 552 POINT MUTATIONS IN THE DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE GENE CONFER RESISTANCE TO PYRIMETHAMINE IN FALCIPARUM MALARIA, David S. Peterson, David Walliker and Thomas E. Wellems, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.
Analysis of a genetic cross between a pyrimethamine-resistant clone (HB3) and a pyrimethamine-sensitive clone (3D7) of Plasmodium falciparum indicates that resistance to the antifolate pyrimethamine results from a point mutation in the dihydrofolate reductase-thymidylate synthase gene. Parasites having a mutation at position 108 from Thr/Ser to Asn are resistant to the drug. All progeny have parental levels of sensitivity to pyrimethamine, suggesting that in clone HB3 resistance is determined solely by inheritance of the DHFR-TS gene. Nucleotide sequence analysis of independent parasite isolates from Southeast Asia, Africa and South America reveals additional point mutations (Asn-51 to Ile-51, and Cys-59 to Arg-59) in the DHFR coding region that are associated with increased pyrimethamine resistance. Comparison with the crystallographic structure of DHFR determined for other organisms suggests that these point mutations occur where pyrimethamine contacts the enzyme and may act by inhibiting binding of the drug.

O 553 **IDENTIFICATION OF A COMMON EPITOPE ON TRYPANOSOMA CRUZI AND MAMMALIAN NERVOUS TISSUE.** **Klaus Petry, Sen-itiroh Hakomori and Harvey Eisen, Fred Hutchinson Cancer Research Center M723, Seattle, WA 98104**

Chronic Chagas' disease, caused by infection with T. cruzi, is characterized by lesions of many organs including nervous system and muscle. It has been suggested that this pathology is caused by autoimmunity provoked by the parasite. Although the mechanism by which this autoimmunity is induced is not understood, the presence of cross-reactive determinants between the parasite and the mammalian host has been suggested.

We have identified monoclonal antibodies (MABs) that react with T. cruzi and mammalian brain cells. Preliminary experiments indicated that many of these cross-reactive antibodies recognize lipid antigens. One of these MABs, VESP 8.2, which cross-reacts with sialidase treated T. cruzi, glial cells and sciatic nerve recognizes an epitope common to two families of glycolipids: galactosyl-ceramide (isolated from both parasite and mammalian brain), and sulfo-galactosyl-ceramide (isolated from the brain). The antigenic members of these families appear to contain specifically 2-hydroxy fatty acids. The antigens isolated from T. cruzi were not found in the media components used for the parasite culture indicating that the antigens were of parasite origin rather than scavenged from the culture medium.

O 554 PARAMYOSIN IS A MAJOR *S. MANSONI* ALLERGEN, Raymond J. Pierce, Philippe Boutin and André Capron, Unité Mixte INSERM U 167 - CNRS 624, Institut Pasteur, 59019-Lille, France.

Our interest in the role of the IgE response in protection against schistosomiasis led us to attempt to clone molecules recognized by IgE in infection sera. Three such clones were obtained, one of which had a large insert (2600 bp) and synthesized a β-galactosidase fusion protein of about 200 kDa. The insert contained two internal EcoR1 sites and the central fragment of 1317 bp was completely homologous to the published partial sequence of *S. mansoni* paramyosin[1]. The complete insert codes for a 99 kDa polypeptide but both the N- and C-termini are absent. The expressed fusion protein was recognized by IgE in the sera of infected rats and humans. Furthermore, immunization of rats with the fusion protein produced an IgE response to native paramyosin. It would therefore seem that while paramyosin is the target of vaccine induced, cell-mediated resistance to schistosomiasis in mice[2], it induces an anaphylactic antibody response in rats and man. The possible role of anti-paramyosin IgE in host defence is under investigation.

1. Lanar, D.E., Pearce, E.J., James, S.L. and Sher, A. (1986) Science, 234, 593.
2. Pearce, E.J., James, S.L., Hieny, S., Lanar, D. and Sher, A. (1988) Proc. Natl. Acad. Sci. USA, 85, 5678.

O 555 **IN VITRO PROLIFERATION OF Plasmodium falciparum IS SELECTIVELY INHIBITED BY 5-FLUOROOROTATE, Pradipsinh K. Rathod, Ashok Khatri, Institute for Biomolecular Studies, Department of Biology, The Catholic University of America, Washington D.C. 20064, and Ted Hubbert, Wilbur K. Milhous, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington D.C. 20307.**

Unlike mammalian cells, malarial parasites utilize exogenous orotic acid more efficiently than pyrimidines such as uracil and uridine. The in vitro susceptibility of P. falciparum to 5-fluoroorotate, 5-fluorouracil, 5-fluorouridine, and 5-fluoro 2'-deoxyuridine was compared to the toxicity of these drugs towards human fibrosarcoma cells in culture. The highest antimalarial activity was observed with 5-fluoroorotate (IC_{50}=6nM). Uridine did not protect malarial parasites from this drug. In contrast, 5-fluoroorotate was the least toxic drug to the human cells (IC_{50}=10uM). Furthermore, a combination of 5-fluoroorotate and uridine resulted in a 3-fold decrease in toxicity to mammalian cells. A chloroquine-resistant P. falciparum clone that showed cross-resistance to pyramethamine, sulfadoxine, and quinine, was as vulnerable to 5-fluoroorotate as a standard drug-sensitive clone.

O 556 FOLATE METABOLISM AND DRUG RESISTANCE IN TOXOPLASMA GONDII. *David S. Roos, and Robert T. Schimke. Department of Biological Sciences, Stanford University, Stanford, CA 94305*

We have investigated several aspects of folate metabolism in the coccidian parasite *Toxoplasma gondii*. Clinical therapy of acute toxoplasmosis typically involves treatment with a combination of folate antagonists (such as pyrimethamine) and sulfonamides (e.g. sulfadiazine, an analog of the dihydrofolate precursor *para*-amino benzoic acid). These two drugs have a profound synergistic effect, but the precise 'isobologram' patterns of interactions between them remain unknown. We have therefore developed a simple microassay appropriate for the screening of parasiticidal agents *in vitro*. This assay has demonstrated a surprisingly large range of drug sensitivity in naturally occuring populations of *T. gondii*, including isolates derived from opportunistic infections associated with AIDS. Drug sensitivity has also been examined in a variety of stable pyrimethamine-resistant parasites, including both natural isolates and mutants selected in the laboratory. The most highly altered of these mutants are >100-fold resistant to the toxic effects of pyrimethamine, and exhibit cross-resistance to a wide variety of other antifolates, including both water-soluble and lipophilic analogs. Molecular cloning of the wild-type *T. gondii* dihydrofolate reductase gene reveals a fused enzyme bearing homology to thymidylate synthase as well as DHFR, as found in several other protozoa. Sequence analysis predicts a protein distinct in many respects from other known DHFR/TS genes, but with greatest similarity to *Plasmodium*. While the molecular basis of drug resistance in the mutant parasites is not yet known, it appears not to involve amplification or rearrangement of the DHFR/TS gene.

O 557 MOLECULAR ANALYSIS OF BENZIMIDAZOLE RESISTANCE IN THE PARASITIC NEMATODE HAEMONCHUS CONTORTUS.

M.H. Roos, J.H. Boersema, P.R. Nilsson, F.H.M. Borgsteede, M. Taylor and E.J. Ruitenberg (resp. Division of Helminthology and Entomology, School of Veterinary Medicine, University of Utrecht, The Netherlands; Central Veterinary Institute, Lelystad, The Netherlands; Central Veterinary Institute, Weybridge, U.K.; National Insitute of Public Health and Environmental Protection, Bilthoven, The Netherlands.)

The sheep intestinal parasite H. contortus and its resistance to benzimidazoles was characterized because resistance develops regularly, these drugs are widely used and new derivatives are being developed. Benzimidazoles are reported to interfere with microtubule assembly and therefore tubulin genes from normal and resistant populations were analyzed by Southern blotting. Three normal populations from different geographical locations, were polymorphic for beta-tubulin, 2-6 bands were visible, depending on the restriction enzyme used. The different populations could be discriminated according to the banding pattern. Three resistant populations, also from different locations, showed 1-2 bands. The bands that were found in the resistant populations were already present in a low concentration in the normal strains. An in vitro selection experiment on the eggs of a normal population showed that after two generations the population had become resistant and that the tubulin DNA bands were reduced from 6 to 1. These results indicate that benzimidazole resistance may be the result from selection of worms, with a specific beta-tubulin variant, that are already present in the population.

O 558 A CYSTEINE PROTEINASE OF P. FALCIPARUM TROPHOZOITES RESEMBLES CATHEPSIN L AND IS A POTENTIAL TARGET FOR CHEMOTHERAPY, Philip J. Rosenthal[1], James H. McKerrow[2], David Rasnick[3], and James H. Leech[1], [1]Dept of Medicine, San Francisco General Hospital, San Francisco, CA 94110, [2]Dept of Pathology, U. California, San Francisco, CA 94143, and [3]Enzyme Systems Products, Dublin, CA 94568. Trophozoites of P. falciparum obtain free amino acids for protein synthesis by degrading host erythrocyte hemoglobin in an acidic food vacuole (FV). We previously reported that leupeptin and E-64, two inhibitors of the cysteine class of proteinases, blocked hemoglobin degradation in the trophozoite FV, and we identified an Mr 28,000 trophozoite cysteine proteinase (TP) as a potential FV hemoglobinase. We have now further characterized the TP in order to help determine its function and to investigate its potential as a target of antimalarial chemotherapy. By determining the kinetics of hydrolysis of a variety of fluorogenic peptides, we found that the TP resembled the lysosomal cysteine proteinases cathepsin B and cathepsin L in terms of pH optimum, inhibitor sensitivity, and substrate preference. The TP had a pH optimum of 5.5-6.0, near the pH of the FV (5.2-5.4), and was efficiently inhibited by highly specific diazomethylketone and fluoromethylketone inhibitors of cathepsin B and cathepsin L. The TP preferred substrates with arginine adjacent to hydrophobic amino acids (Z-Val-Leu-Arg-AMC, Z-Phe-Arg-AMC), as does cathepsin L. The similarities between the TP and the lysosomal enzymes support our hypothesis that the trophozoite proteinase is a FV hemoglobinase, since the FV resembles mammalian secondary lysosomes in its pH and its biological role as a site of protein degradation. Micromolar concentrations of the fluoromethylketone inhibitor Z-Phe-Ala-CH_2F blocked the degradation of hemoglobin in the trophozoite FV and prevented parasite multiplication. In previous studies much higher concentrations of the inhibitor were not toxic for mice. These results suggest that specific inhibitors of the TP may have potential as antimalarial drugs.

O 559 IDENTIFICATION OF DEVELOPMENTALLY REGULATED CALMODULIN-BINDING PROTEINS IN *TRYPANOSOMA BRUCEI*, Larry Ruben, Nasser G. Haghighat and Allen Campbell, Department of Biological Sciences, Southern Methodist University, Dallas, TX 75275.
Calmodulin is a critical component of cellular regulatory pathways. Calmodulin has no intrinsic enzymatic activity but associates with calcium and mediates a wide range of cellular activities. In the present study, an [^{125}I]trypanosome calmodulin overlay procedure was used to detect calmodulin-binding proteins (CaMBPs) with Mr of 126, 106 and 65 K in homogenates of slender bloodstream forms of *T. brucei*. Competition assays with excess unlabeled bovine brain or trypanosome calmodulins demonstrated that the CaMBPs could associate with calmodulin from either source. Homogenates of *T. evansi* exhibited the same pattern of calmodulin-binding acitivity as *T. brucei* slender bloodstream forms. However *T. cruzi*, *L. tarentolae* and mouse brain contained distinct patterns of calmodulin-binding activity. The pattern observed for mouse brain was the same whether [^{125}I]trypanosome or [^{125}I]brain calmodulin was used as a probe. Therefore trypanosome calmodulin interacts with host tissue in the same manner as host calmodulin. When procyclic culture forms were examined for CaMBPs, the 126 and 106 kDa proteins were absent. The developmentally regulated CaMBPs from *T. brucei* were in the 10,000 xg pellet. The only process currently known to exhibit the same pattern of developmental change reported here for CaMBPs involves biosynthesis and transport of variable surface glycoprotein. We conclude that sensitivity towards calmodulin varies throughout the trypanosome life cycle, in part due to changes in the cellular complement of CaMBPs. Novel features of calcium regulatory pathways in *T. brucei* may ultimately prove useful in the design of diagnostics or new therapies. Supported by NIH grant # AI24627

O 560 CHARACTERIZATION OF THE PHOSPHOLIPASE INVOLVED IN *TOXOPLASMA GONDII* HOST CELL PENETRATION, Linda D. Saffer and Joseph D. Schwartzman, Department of Pathology University of Virginia School of Medicine, Charlottesville, VA 22908. A parasite phospholipase A (PLA) may be involved in host penetration by tachyzoites of *T. gondii*. Exogenous reptilian PLA2 increased host cell penetration by *T. gondii* and anti-PLA2 (anti-snake-venom horse serum) decreased penetration in assays in which intracellular parasites were quantitated by the selective incorporation of tritiated uracil; both effects were dose dependent. Two PLA2 inhibitors, p-bromophenacyl bromide and nordihydroguaiaretic acid, blocked penetration when parasites were exposed to the inhibitors, but had little effect when host cells were pretreated with the inhibitor, which suggests that a parasite specific PLA was inhibited. In order to identify whether PLA acts on the lipids of the host membrane or the parasite membrane, we labeled either parasite or host fibroblast lipids with ^{14}C acetate. Fibroblasts were then exposed to infectious parasites or to sonic extracts of *T. gondii*. The lipids were then extracted from fibroblasts or purified *T. gondii* and separated by thin layer chromatography. Activity specific to particular lipid components was quantitated by scintillation spectroscopy of fractions of the silica matrix corresponding to co-chromatographed controls. Preliminary results show that *T. gondii* and human fibroblasts have similar lipid labelling patterns, and that parasites may salvage lipids from the host. The activity of parasite PLA2 is low in unfractionated extracts.

O 561 GAMMA-IFN AND H_2O_2 RESPONSES IN SUSCEPTIBLE AND RESISTANT MICE DURING LETHAL AND NON-LETHAL RODENT MALARIA. Hannah L. Shear and Roopa Srinavasan. Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY 10016. The ability of malaria-susceptible (Balb/c ByJ) and resistant (CBA/J) mice to respond to lethal (PyL) and non-lethal (PyNL) variants of Plasmodium yoelii 17X was studied. Susceptible mice responded to non-lethal P. yoelii infection with an early burst of gamma-IFN and H_2O_2, a decline as parasitemia increased and a return to control levels as the infection was cleared. Infection of Balb/c ByJ mice with PyL resulted only in an early H_2O_2 response, a decline, and no recovery of activity. All the animals succumbed to PyL. Resistant CBA/J mice responded to both lethal and non-lethal Py with an early burst of both gamma-IFN and H_2O_2 and a decline as parasitemia increased. However, there was a recovery of both activities, to higher than control levels in the case of gamma-IFN, as the infections were cleared. In addition, mice injected with recombinant mouse gamma-IFN (Genentech, Inc.) also showed greatly enhanced secretion of H_2O_2 by macrophages. These data suggest that gamma-IFN may play an important role in controlling malaria infections, perhaps by activating macrophages to release H_2O_2 and other reactive oxygen intermediates.

O 562 ANTI-BAND 3 AUTOANTIBODIES RECOGNIZE A HIGH MOLECULAR WEIGHT PROTEIN ON THE SURFACE OF KNOBBY ERYTHROCYTES INFECTED WITH PLASMODIUM FALCIPARUM, Irwin W. Sherman and Enrique Winograd, Department of Biology, University of California, Riverside, CA 92521, U.S.A. In vivo infections with the human malaria parasite Plasmodium falciparum are characterized by the absence of mature forms of the parasite in the peripheral circulation. This phenomenon results from binding of infected erythrocytes to the post-capillary venular endothelium of the deep tissues. Attachment of parasitized erythrocytes is mediated via membrane excrescences or knobs. By transmission electron microscopy knobs appear as electron dense masses lying below a localized protrusion of the red cell. Erythrocytes infected with the knobby variant of P. falciparum (FCR-3 strain) express significant amounts of senescent antigen, as indicated by the fact that naturally occuring anti-band 3 autoantibodies bind specifically to these cells. The autoantibodies immunoprecipitated a > 240 kDa protein from SDS extracts of surface iodinated infected erythrocytes. The > 240 kDa antigen was associated only with erythrocytes infected with a knobby variant and not knobless ones; the antigen was removed by trypsin treatment of intact infected cells. By two-dimensional peptide map analysis the > 240 kDa antigen was found to be structurally related to the human erythrocyte anion transporter, band 3. We propose that the > 240 kDa antigen results from the aggregation of a truncated form of band 3 in the region of the knob. This antigen resembles, in several of its properties, the surface protein, Pf EMP 1, found in cytoadherent lines of P. falciparum. (This work was supported by a grant (AI-21251) from the National Institutes of Health.)

O 563 IMMUNOSUPPRESSION IN MURINE TRYPANOSOMA BRUCEI INFECTION: THE ROLE OF SUPPRESSIVE MACROPHAGES, Jeremy M. Sternberg, Norbert K. Borowy, Dagmar Schreiber and Peter Overath, Max-Planck-Institut für Biologie, Corrensstraße 38, D7400 Tübingen, FRG.

Immunosuppression is a major pathological consequence of trypanosomiasis. Using a mouse model we have shown the macrophage to be a key mediator in this process, both *in vivo* and *in vitro*. Infection of mice of the C3H.He, BALB/C or C57Bl/6 strains with *T.brucei* (AnTat1.1) resulted in impaired immune responses from day 3 onwards as measured by the reduction of Con-A stimulated proliferation in spleen cell populations. Peritoneal or splenic adherent cell populations, consisting predominantly of macrophages, caused a 60-80% reduction of the Con-A response in spleen cells from syngeneic recipients, 3-4 days after transfer *in vivo*. Experiments with labelled cells showed that a significant proportion of transferred macrophages localised in recipient spleens within this time period. Adherent peritoneal cells from irradiated or athymic mice were equally suppressive, arguing against a role for contaminating T and B cells or antibody mediated phagocytosis. Spleen cells from infected mice reduced the proliferative response of spleen cells from uninfected mice upon co-cultivation *in vitro*. This dominant suppressive effect was abolished if macrophages were first eliminated from spleen cells derived from infected mice by treatment with L-leucine methyl ester. This treatment left less than 1% non-specific esterase$^+$ cells. Also, macrophage depleted spleen cells from infected mice responded normally to Con-A following reconstitution with splenic adherent cells from naive mice as a source of accessory cells. These findings suggest that *T.brucei* infection changes the properties of macrophages to a state which enables them to actively suppress immune responses.

O 564 BANCROFTIAN FILARIAL INFECTION IN MICE: PROTECTIVE IMMUNE RESPONSES, D. Subrahmanyam, G.R. Rajasekariah, I.M. Monteiro and A. Netto, Research Centre, Hindustan Ciba-Geigy Ltd., Bombay, India and Ciba-Geigy Ltd. Basel, Switzerland.

Epidemiologic observations suggest the existence of a segment of population, in areas endemic to filariasis, not acquiring the infection despite repeated exposures to it. The mechanism by which such resistance is acquired needs to be understood for devising possible intervention methods against the infection. In this context, we have examined the possibilities of inducing resistance to larval infection in a BALB/c mouse-Wuchereria bancrofti model. Mice were sensitized repeatedly at periodic intervals with live infective larvae of W. bancrofti (WbL3). The sensitized animals were challenged subcutaneously with live L3 enclosed in micropore chambers. The repeated trickle infections with L3 led to development of a strong protective immune response in the mice with over 90% of the challenging larvae killed by antibody dependent cell-mediated immune reactions. Similar immune responses were also seen when mice were immunized with microfilarial extracts. This suggested that dead parasites can induce protection against the infection and demonstrated a sharing of antigens between microfilariae and infective larvae. The kinetics of immune responses have been studied in the animals and these will be presented and discussed.

O 565 CHARACTERIZATION OF A cDNA CLONE ENCODING THE TRYPANOTHIONE DISULPHIDE REDUCTASE OF Leishmania donovani, Martin C. Taylor, Caroline J. Chapman, John M. Kelly, Alan H. Fairlamb and Michael A. Miles, Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT. A cDNA clone (LTR1) was isolated from a Leishmania donovani (HU3) λgt11 expression library using an antiserum raised against trypanothione disulphide reductase (TR) purified from Crithidia fasciculata. The LTR1 insert (320bp) was subcloned and sequenced; the inferred amino acid sequence showed 73% identity with the corresponding region of Trypanosoma congolense TR. Southern blots using the LTR1 probe indicated a single copy gene, and on northern blots a single mRNA species was detected in both promastigotes and amastigotes. Although this probe did not cross-hybridise with Trypanosoma cruzi DNA an oligonucleotide probe derived from a conserved region of the sequence hybridised to T. cruzi, L. mexicana and L. arabica RNA. The LTR1 probe was used to isolate a cDNA clone (LTR3) from a λgt10 library. Sequence analysis indicated that the LTR3 insert (1.8kb) spanned 90% of the TR coding region.

O 566 STRUCTURE-ACTIVITY STUDIES ON THE EFFECTS OF NOVEL PENTAMIDINE DERIVATIVES AGAINST PARASITIC MICROORGANISMS. Richard R. Tidwell, Constance A. Bell, Susan K. Jones, James Edwin Hall, Vijitha Linga, Margaret A. Allen, University of North Carolina at Chapel Hill, NC 27599, Dennis E. Kyle, Max Grogl, Division of Experimental Therapeutics, WRAIR, Washington, DC, 20307-5100.

Pentamidine has long been used to effectively treat African trypanosomiasis and antimony resistant leishmaniasis and has recently proven effective in treatment of AIDS-related Pneumocystis carinii pneumonia (PCP). Pentamidine, however, is toxic and its mode of action is unknown. We have begun to examine structure-activity relationships of pentamidine analogs with respect to their toxic effects and activity against Giardia lamblia, Leishmania mexicana amazonensis, and P. carinii. Twenty-five analogs were synthesized and tested against G. lamblia and L. m. amazonensis in vitro and in immunosuppressed rats with PCP. Pentamidine was found to have moderate activity against P. carinii and G. lamblia, while some of the less toxic analogs had pronounced antiparasitic activity relative to the parent drug. With respect to anti-leishmanial activity, only a few of the derivatives were found to have even a modest increase in potency over pentamidine. Correlations between drug uptake, DNA binding, and protease inhibition with antiparasitic activity suggest possible sites of action of diamidines.

O 567 **Antisense oligonucleotides targeted to the mini-exon of *Trypanosoma brucei* : in vitro studies with nuclease resistant oligomers.** Jean.-Jacques Toulmé, Philippe Verspieren, David Shire* and Nadine Loreau, INSERM U201, MNHN, 43 rue Cuvier, F-75005 Paris and * Sanofi Elf BioRecherches, F-31328 Labège, France. It was previously demonstrated that oligodeoxynucleotides, complementary to the mini-exon sequence present at the 5' end of all mRNAs, linked to an acridine derivative were able to kill trypanosomes in culture (Verspieren et al. (1987) Gene, 61, 307). However the oligonucleotides were rapidly degraded by DNases both in the growth medium and in the cells. Chemical modifications have been introduced into the backbone of the anti-mini-exon molecules in order to make them resistant to nucleases. Both methylphosphonate (MP) and phosphorothioate (PS) analogs have been synthesized. These derivatives are able to bind to their target although with a reduced affinity as compared to unmodified oligomers. Whereas PS-oligomers inhibited *in vitro* translation of *T. brucei* mRNA, MP-derivatives did not prevent protein synthesis. This is very likely due to the sensitivity of oligonucleotide-mRNA hybrids to RNaseH. PS-derivatives induced degradation of mRNA; in contrast MP-oligomer-mRNA hybrids were not a substrate for this enzyme. This suggests that degradation of the target-RNA is required for inhibition by antisense oligodeoxynucleotides even at the initiation step of translation. (This work was supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases).

O 568 AUGMENTED EXPRESSION OF ORNITHINE DECARBOXYLASE IN MUTANT LEISHMANIA DONOVANI RESISTANT TO DIFLUOROMETHYLORNITHINE, Buddy Ullman, Department of Biochemistry, The Oregon Health Sciences University, School of Medicine, Portland, OR 97201. A strain of mutant Leishmania donovani promastigotes has been generated by virtue of its ability to propagate in growth medium containing incremental concentrations of alpha-difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase (ODC) activity. This strain, DFMO-10, was over 100-fold less sensitive to growth inhibition by DFMO and was cross-resistant to a spectrum of other ODC inhibitors. Unlike previously isolated DFMO-resistant Trypanosoma brucei, the DFMO-10 cells accumulated or transported [^{3}H]DFMO and a spectrum of amino acids at comparative rates. DFMO-10 cells after several days growth in the absence of DFMO contained up to 15-fold greater levels of ODC than wild type parental cells. The augmented ODC in the mutant cells appeared kinetically normal with respect to substrate affinity and sensitivity to inactivation by DFMO. Incubation of extracts of mutant cells, but not wild type cells, for one hour with [^{3}H]DFMO resulted in the covalent labeling of a polypeptide, presumably ODC, which migrated on SDS polyacrylamide gels with a molecular weight of 76,000 daltons. Levels of putrescine, the product of ODC, were also vastly augmented in mutant cells after several days incubation in the absence of the drug, whereas, ornithine and arginine levels were very low. Spermidine levels appeared unchanged. In the absence of prolonged selective pressure, the growth resistance to DFMO, the ODC levels, and the putrescine content of the DFMO-10 cells returned to those of wild type cells, indicating that the mutant phenotype of the DFMO-resistant Leishmania was unstable.

O 569 Translation inhibition of trypanosomatidae mRNAs by antisense oligodeoxynucleotides. Philippe Verspieren, Nadine Loreau and Jean.-Jacques Toulmé, INSERM U201, MNHN, 43 rue Cuvier, F-75005 Paris, France. Hybrid-arrested translation of *T. brucei* mRNA was observed in the presence of oligonucleotides complementary to the mini-exon sequence (Cornelissen et al. (1986) Nucleic Acids Res., 14, 5605). We investigated the effects of a series of Anti-Mini-EXon Oligonucleotides (AMEXOs) on the *in vitro* protein synthesis of mRNAs from *T. brucei, T. vivax, L. enrietti and L. mexicana.* We also determined the affinity of these AMEXOs for the different RNAs by thermal elution of filter-bound complexes. The following conclusions were drawn; i) A relationship was derived between affinity and (length/G+C content) of the oligonucleotide. ii) In the case of the *L.e.* mini-exon, discrepancies between expected and measured affinities could be explained by the formation of a hairpin structure. iii) This technique allowed to predict the level of homology between known (*T.b., T.v., L.e.*) and unknown (*L.m.*) mini-exon sequences. iv) In most cases the efficiency of translation inhibition was correlated to the affinity of AMEXOs for their target. This work was supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

O 570 PROTECTIVE ANTI-SPOROZOITE ANTIBODIES: ISI ASSAY USING RIBOSOMAL RNA PROBES, Jindong Zhu, Altaf Lal, Angela Appiah, Michael R. Hollingdale, Biomedical Research Institute, Rockville, MD 20852.
The inhibition of sporozoite invasion (ISI) of human hepatoma (HepG2-A16) cells assay has been suggested as an in vitro assay that measures protective anti-sporozoite anti-bodies elicited in humans by irradiated sporozoites or Plasmodium falciparum CS subunit vaccines. Since CS vaccines are undergoing human trials, the ISI assay has become widely used. However, a major limitation is the use of microscopy to determine the reduction of invaded sporozoites in the presence of immune antibodies. Sporozoites are added to HepG2-A16 cells in the presence of dilutions of immune antibodies and ISI activity is expressed either as the percent reduction in invasion at that dilution, or as ISI_{50}, that concentration that reduces invasion by 50%. We have now developed stage-specific rRNA probes that specifically react with invaded sporozoites but not with attached but uninvaded sporozoites. The technique involves one step extraction of total RNA and hybridization to an oligo-DNA probe complementing to small subunit Plasmodium rRNA. As few as 10-50 invaded sporozoites can be detected, whereas rRNA from attached sporozoites (using cytochalsin B) does not hybridize. The ISI of Mab's to CS proteins was similar by microscopy or rRNA hybridization. Use of rRNA probes is faster and more accurate than microscopy, allowing more sera to be tested. The degree of hybridization of the rRNA probes increased during growth of liver stage parasites suggesting that such probes will also be useful in quantitating hepatic parasitemia in vitro and in vivo in response to other immune factors such as cytokines, and drugs, as well as for diagnosis of blood stage infections.

O 600 MUTATIONAL ANALYSIS OF GLYCOSOMAL IMPORT OF PGK IN TRYPANOSOMA BRUCEI, Keith Alexander, Teresa Hill, *Harry F. Dovey, *Ching C. Wang and Marilyn Parsons, Seattle Biomedical Res. Inst., 4 Nickerson, Seattle, WA 98109, *Dept. Pharmaceut. Chem., U. California, San Francisco, CA 94143. In the bloodstream stage of T. brucei the first seven enzymes of glycolysis are contained within microbody organelles called glycosomes. The mechanisms by which these organelles identify and import proteins are unclear. We have chosen to study the localization of phosphoglycerate kinase because it is possible to compare the amino acid sequences of the glycosomal form (gPGK) with the cytoplasmic form found in the procyclic parasite. The amino acid residues that are different must by default contain all or part of the targeting signal. Starting from the cloned gPGK gene, we have made a number of deletion mutants. Because of possible changes in the tertiary structures of the mutant proteins, a second series of mutants were constructed by replacing parts of the gPGK sequence with their counterparts from the cytoplasmic gene. The modified genes have been transcribed and translated in vitro and tested in an uptake assay system using purified glycosomes (Dovey et al, PNAS 1988). Several gPGK fragments specifically interact with glycosomes. However, preliminary results suggest that the import signal or signals are complex and may require proper protein folding for functional presentation.

O 601 CHARACTERIZATION OF 3'-NUCLEOTIDASE IN TRYPANOSOMA BRUCEI RHODESIENSE. Paul A. Bates, Department of Zoology, University of Glasgow, Glasgow, U.K. Variant surface glycoproteins (VSGs) have been the subjects of intensive research in African trypanosomes but investigations of the basic biochemistry of the surface membrane have been relatively few. Identification of essential housekeeping molecules of the trypanosome surface, such as enzymes and transporters, may help in the rational design of drugs and vaccines. 3'-nucleotidase, a unique parasite enzyme, was investigated for suitability as a trypanosome surface membrane marker. Membrane ghosts were prepared from bloodstream trypanosomes by hypotonic lysis and differential centrifugation. In the presence of the protease inhibitors leupeptin and TLCK the 3'-nucleotidase activity sedimented with the membrane fraction. However, if inhibitors were omitted the enzyme remained largely in the soluble fraction, indicating that endogenous proteolytic activity had released an enzymatically active fragment from the membranes. Incubation of the crude membrane fraction with protease inhibitors at 37°C for 5 min resulted in the activation of VSG lipase and the release of soluble VSG, as assessed by SDS-PAGE. Under these conditions 3'nucleotidase activity remained membrane bound indicating that this enzyme does not possess a lipid membrane anchor capable of cleavage by VSG lipase. Enzyme could be solubilised from membranes by using either of the non-ionic detergents n-octyl glucoside and Triton X-100. This caused a 20% increase in activity due to latency or stimulation. The combination of membrane preparation and detergent solubilisation effected a 4-fold increase in relative specific activity of 3'nucleotidase.

O 602 ZINC SIGNATURE IN THE PROMASTIGOTE SURFACE PROTEASE OF LEISHMANIA, C. Bordier, J. Bouvier, P. Schneider & E. Shaw, BIOKEMA SA, CH-1023 Crissier, Université de Lausanne, CH-1066 Epalinges, Friedrich Miescher Institut, CH-4002 Basel, Switzerland. Most species of Leishmania express a very abundant surface membrane glycoprotein of approximately 63 kDa. This glycoprotein has an endopeptidase activity and is anchored in the membrane of the parasite by a glycosyl phosphatidylinositol. Recent investigations have shown that the protease contains zinc and can be biosynthetically labeled with the radioactive metal. The presence of zinc in the active site of the enzyme is confirmed by the homology existing between a sequence of amino acids present in the active site of thermolysin and of various mammalian endopeptidases and a short sequence of the Promastigote Surface Protease. The design and synthesis of zinc chelating oligopeptides analogous to substrates of the protease has permitted the identification of inhibitors of the enzyme.

O 603 ZINC SIGNATURE IN THE PROMASTIGOTE SURFACE PROTEASE OF *LEISHMANIA*. J. Bouvier[1,2], P. Schneider[2], R. Etges[2,3], E. Shaw[4] and C. Bordier[2]
[1]Dept. of Pathology, UCSF, San Francisco, CA, [2]Institut de Biochimie, Université de Lausanne, Epalinges, Switzerland, [3]Max Planck Institut, Tübingen, Germany and [4]Friedrich Miescher-Institut, Basel, Switzerland.
Promastigotes of *Leishmania* express a very abundant surface membrane glycoprotein of 63 kDa (gp63). This protein is anchored in the membrane of the parasite by a glycosylphosphatidylinositol moiety and possesses a strong neutral-to-alkaline proteolytic activity. Its role as the principal C3 acceptor, the major surface antigen and vaccine candidate, and as an RGD-bearing ligand contributing to the binding of *Leishmania* to host macrophages has received much attention, but little is known about its proteolytic activity. The protein, now designated as the promastigote surface protease, or PSP, is a conserved enzyme found at the surface of all *Leishmania* species tested so far, as well as on the surface of promastigotes within the midgut of experimentally-infected phlebotomine sandfly vectors. Recent investigations with emission and absorption atomic spectrometries have shown that the protease is a zinc enzyme containing 1 mole of metal per 63 kDa monomer. The presence of zinc in the active site of the enzyme was confirmed by the identity existing between the sequence of the active site of thermolysin (a zinc enzyme with a known 3D structure) and a short sequence of PSP. The substrate specificity of the enzyme is currently under investigation and is still not clear. However,the identification of several amino acids involved in cleaved peptide-bonds has lead to the design and synthesis of a specific zinc chelating oligopeptide analogous to substrates of the enzyme.

O 604 ATP TRANSPORT ACROSS THE PARASITOPHOROUS VACUOLAR AND PLASMA MEMBRANES OF P. FALCIPARUM, Inpyo Choi and Ross B.Mikkelsen, Department of Radiation Oncology, Medical College of Virginia, Richmond, VA 23298.

Previous studies have shown that development of P.falciparum from ring to schizont outside the host erythrocyte requires a cytosolic like ionic medium and extracellular ATP. Bongkreic acid, an inhibitor of mitochondrial ATP:ADP exchange, blocks Plasmodium growth. We have characterized ATP transport using free parasites isolated at the trophozoite stage by immune lysis or N_2-cavitation. Kinetic analysis of [^{3}H]-ATP uptake revealed a K_m of 0.4 mM. Uptake was due to ATP:ADP exchange since [^{3}H]-ADP efflux was dependent on exogenous ATP. ATP influx and ADP efflux were inhibited by atractyloside (K_i=100nM). Analysis of substrate specificity indicated that for ATP influx the adenine ring and triphosphate are recognition components for the transporter. In addition, protein synthesis was measured with free parasites starved of glucose with and without addition of exogenous ATP. Addition of 1.0 mM ATP to starved cultures resulted in recovery of 60% of protein synthetic capacity in a process inhibited by 200 nM atractyloside. These results indicate that uptake of erythrocyte derived ATP by Plasmodium is essential for maintaining maximal rates of protein synthesis.

O 605 INTERACTIONS BETWEEN PLASMODIUM FALCIPARUM ANTIGEN MESA AND THE ERYTHROCYTE MEMBRANE SKELETON. R.L. Coppel, S. Lustigman, R.F.Anders and D.J. Kemp.The Walter and Eliza Hall Institute of Medical Research.

During intraerythrocytic growth of the malaria parasite P.falciparum, proteins elaborated by the parasite are exported to the erythrocyte membrane where they alter the morphological and functional properties of the erythrocyte. These changes in red cell properties occur as a consequence of the interaction between parasite proteins and components of the erythrocyte membrane skeleton. We have described the mature-parasite-infected erythrocyte surface antigen (MESA) of **P.falciparum**, a phosphoprotein of 250-300kDa that varies in size and antigenicity among different parasite isolates (1,2). Synthesized in trophozoites and schizonts, MESA is transported through the erythrocyte cytoplasm to the membrane skeleton. We now show that MESA binds tightly to the erythrocyte protein band 4.1. Band 4.1 of infected cells has an increased level of phosphorylation and in erythrocytes infected with some isolates, band 4.1 increases in apparent molecular weight. The effect of these changes on red cell properties and whether MESA is responsible for phosphorylation changes in band 4.1 is currently under study. In addition, the complete structure of MESA is currently being determined in an effort to define the region responsible for its interactions with band 4.1.

1. Coppel, R.L., Culvenor, J.G., Bianco, A.E., Crewther, P.E., Stahl, H.D., Brown, G.V., Anders, R.F. and Kemp, D.J. (1986).Molec.Biochem.Parasitol. 20:265-277.
2. Coppel, R.L., Lustigman, S., Murray, L. and Anders, R.F. (1988). Molec.Biochem.Parasitol. 31:223-232

O 606 ANALYSIS OF THE rRNA GENES OF *BABESIA BOVIS*, Brian P. Dalrymple, CSIRO Division of Tropical Animal Production Private Bag No. 3, Indooroopilly Queensland Australia 4068. *Babesia Bovis* (strain Samford) appears to contain three or four rRNA operons each present in a single copy in the genome. Two of the operons have been cloned and analysed by restriction enzyme mapping and hybridization. The operons are similar, but not identical, exhibiting some restriction site differences. Homologies between the operons are confined to the rRNA genes and their immediate vicinity. However one of the operons (system 2) is flanked on one side by a region of ~4.5kb present at two sites in the genome, and on the other by a sequence that hybridizes at low stringency to a large number of restriction fragments. The second copy of the two copy sequence has been cloned. It is flanked by regions unrelated to the first copy, and does not appear to be closely linked to an rRNA operon.

O 607 **FATTY ACYLATION OF GIARDIA LAMBLIA PROTEIN.** Siddhartha Das and Frances D. Gillin, Dept. of Pathology, UCSD Medical Center, San Diego, CA. 92103.

Giardia lamblia trophozoites colonize the human small intestine where they are exposed to high concentrations of fatty acids. Recently, we have shown that exposure of cultured trophozoites to free fatty acids, in the presence of a primary bile salt triggers encystation. Therefore, we performed biosynthetic labelling experiments to determine if ^{3}H-myristic acid (MA) or palmitic acid (PA) are incorporated into stage specific proteins of G. lamblia. The major protein labelled with ^{3}H MA or PA migrated at ~49kD in SDS-PAGE. Since the 49kD protein and a number of minor bands (~130, 110, 74, 58, 38, 26kD) were detected in both encysting and control trophozoites labelled with MA or PA, protein acylation does not appear to be related to encystation. Approximately three times more PA is incorporated than MA. Competition experiments suggest that PA and MA may be either incorporated into the same sites or interconverted, since unlabelled MA inhibits the incorporation of ^{3}H PA and vice versa. Neither ^{3}H MA nor PA was removed by treatment with alkali or with the phosphatidylinositol specific phospholipase C from Staph. aureus, showing that the fatty acids are covalently attached by non-ester bonds and suggesting the absence of a glycosylphosphatidylinositol anchor. The 49kD protein is sedimentable and may contain sialic acid or N-acetylglucosamine since it reacts strongly with WGA-peroxidase in nitrocellulose blots and can be isolated with WGA-Sepharose. Because Giardia colonizes an environment rich in fatty acids, protein acylation may be particularly relevant to the host-parasite interaction in giardiasis.

O 608 IMMUNOCHEMICAL COMPARISON AND CHARACTERIZATION OF THE SECRETED ACID PHOSPHATASES OF Leishmania. P.S. Doyle and D.M. Dwyer. Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD 20892.

Promastigotes of all Leishmania species, except L. major, synthesize and secrete an extracellular soluble acid phosphatase (SAcP) capable of dephosphorylating a wide range of substrates and, consequently, of modifying the parasite environment. To assess their relatedness, the SAcP(s) from various species were characterized biochemically and immunochemically. A monospecific rabbit antiserum and monoclonal antibodies made against the L. donovani (1S, clone 2) SAcP immunoprecipitated the enzymatic activities of SAcP(s) from 15 different W.H.O. reference species of Leishmania. SDS-PAGE of immunoprecipitates from metabolically labeled culture supernatants showed marked differences among the SAcP(s) in their relative mobilities and the number of bands resolved. These results indicated that chemical differences exist among the enzymes from various species. Tunicamycin treatment abolished enzymatic activity and resulted in a reduction in apparent molecular weight of the SAcP(s) from three different species indicating the presence of N-linked carbohydrates in the native enzymes. Intracellular amastigotes also synthesize SAcP as the enzyme was immunoprecipitated from metabolically labeled L. donovani-infected macrophage culture supernatants. These cumulative data demonstrate that the SAcP(s) from various species possess conserved antigenic epitopes. As the SAcP is synthesized by amastigotes it might be a suitable target for chemotherapeutic or immunologic intervention.

O 609 **ULTRASTRUCTURAL DEMONSTRATION OF VESICLE-MEDIATED EXPORT OF CYST WALL ANTIGENS OF GIARDIA LAMBLIA.** David S. Reiner, Michael McCaffrey*, and Frances D. Gillin, Dept. of Pathol., UCSD Medical Center, San Diego, CA 92103 and *Dept. of Biol., SDSU.

The Giardia lamblia cyst wall is essential to transmission of giardiasis since it is responsible for cyst survival outside the host. Nevertheless, nothing is known at the ultrastructural level of how the encysting trophozoite lays down the extracellular cyst wall. This is particularly interesting in view of the reported absence of a Golgi apparatus in Giardia. We have induced G. lamblia to encyst in vitro and have reported the appearance early in encystation of specific vesicles (ESV) which contain cyst wall antigens. Ultrastructural studies now show that the ESV are large (~1.5 to 3 micron diameter), membrane-bounded, electron dense or osmiophilic vesicles which frequently protrude from the plasma membrane of encysting cells. ESV are rarely observed in non-encysting cells. Frozen section immuno EM confirms that cyst antigens are concentrated within the ESV as well as the nascent cyst wall. The idea that vesicle contents are released to the developing wall by exocytosis is supported by the observation of fusion between the membranes of protruding immunogold-labelled ESV and the plasma membrane. To begin to determine how ESV are assembled, we studied cells at 5 to 8 hour of encystation, when cyst antigens can already be detected by immunoblot, but few ESV are visible. At this stage, cyst antigens are associated with endoplasmic reticulum and sometimes with membrane stacks which resemble a Golgi structure. These studies support the hypothesis that an ER-->(Golgi?)-->ESV pathway may be assembled for export of cyst wall antigens.

O 610 A new blood stage antigen of Plasmodium falciparum transported to the erythrocyte surface

E. Hundt, B. Knapp and H.A. Küpper, Dept. of Molecular Biology, Behringwerke AG, 3550 Marburg, F.R.G.

By screening of a λgt11 library from P.falciparum genomic DNA with an antiserum raised against the 41 kD protein band, which confers protective immunity to monkeys (1), a clone was isolated coding for a crossreacting antigen. The insert was used to identify two genomic fragments which carry the entire gene sequence. We present the complete nucleotide and deduced amino acid sequence. The protein encoded contains no repetitive elements, but is characterized by a signal sequence preceded by a charged sequence at the amino terminus as well as by two further hydrophobic segments assumed to be membrane anchor sequences. Antibodies against the expression product of a fragment comprising 70% of the coding region, which strongly reacts with immune sera, inhibit P.falciparum growth in vitro. Western blot analysis revealed a 29 kD schizont antigen, which could be localized on the schizont membrane, on the membrane of parasitized erythrocytes as well as on "Maurer's clefts" by immunoelectronmicroscopy.

(1) L.H. Perrin et al. (1985) J.Clin.Invest. 75, 1718-1721.

O 611 CLONING OF A MEMBRANE-ASSOCIATED ANTIGEN cDNA FROM SCHISTOSOMA MANSONI:HOMOLOGY WITH VERTEBRATE CALPAINS, Steve Karcz and Ron B. Podesta, Cell Sciences Laboratory, Department of Zoology, University of Western Ontario, London, Ontario, Canada, N6A 5B7.

Calpains are calcium-activated neutral thiol proteases found in a variety of vertebrate cells. Two isoforms have been identified which differ in their requirements for activation by calcium. These enzymes have been found to be associated with membranes at sites of cell-cell and cell-substrate adhesion and have been implicated in cytoskeletal reorganization in some cell types.

We have screened an S. mansoni cDNA expression library constructed in the vector λ ZAP using an antiserum raised against apical membrane proteins from adult parasites. In an initial screen of two million recombinant phage, twenty one clones were plaque-purified. Eight clones with different insert sizes were studied further and five recombinants from this panel were found to contain related sequences by hybridization. Sequence analysis of the longest of these related clones (2.4 kb), predicted an open reading frame whose amino acid sequence exhibited 40-50% homology with the deduced sequence of chicken, rabbit, and human calpains. Comparison of the secondary structure and hydropathy profiles of the schistosome and vertebrate sequences also suggested a high degree of structural relatedness. The schistosome sequence shared a similar domain structure with its' vertebrate counterparts, including a C-terminal calcium-binding domain (E-F hand structure). These structural similarities and localization to membranes raise the possibility that the schistosome protein may also play a role in calcium-dependent cytoskeletal-membrane interactions within the tegument of the parasite.

O 612 THE SYNTHESIS AND TURNOVER OF THE MAJOR SURFACE PROTEIN OF *BRUGIA MALAYI*.

Gek-Eng Kwan-Lim, Murray E. Selkirk*, William F. Gregory and Rick M.Maizels, Depts of Pure and Applied Biology and *Biochemistry, Imperial College of Science Technology and Medicine, London SW7 2BB, UK.

The 29K molecule is a major surface glycoprotein of *Brugia* species. This molecule is present on the surface of both L4 and adult worms but absent from less mature stages. It is highly immunogenic and contains two N-linked oligosaccharides together with a single exposed trypsin site on the surface of adult worms. In cell-free translation of adult mRNA, a 40K product is synthesized; in the presence of microsomal membranes this is cleaved at a signal peptidase site to a 38K peptide. In living worms, a 32K transient precusor is seen in metabolic pulse-chase experiments and this is processed into the 29K molecule within 30 minutes of synthesis. Localising this molecule by immuno-electron microscopy in adult worms, it is found to be synthesized in the hypodermis, concentrated on the hypodermal membrane and distributed throughout the cuticle. This protein is also present on the basement membrane of the basal lamella of the gut. A 29K molecule is a major component of ES released *in vitro* into culture medium by adult worms; this 29K shows identical 2D gel migration and glycosidase sensitivity to the surface 29K. Metabolic *in vitro* pulse-chase experiments confirm that the 29K is turned over from adult worms and can be detected in culture medium within 4 hours of labelling.

O 613 MOLECULAR CLONING AND CHARACTERIZATION OF PC96, AN ERYTHROCYTE MEMBRANE ANTIGEN FROM PLASMODIUM CHABAUDI ADAMI, Carole A. Long, J.P.D. Goldring, William P. Weidanz, C. Wanidworanun and Hannah L. Shear, Department of Microbiology and Immunology, Hahnemann University, Phila., PA 19102 and Dept. of Parasitology, New York Univ., New York, NY 10016. A 96kd malarial antigen (Pc96) has been reported to be associated with the plasma membrane of erythrocytes infected with the murine malarial parasite P. chabaudi adami. Immunization with affinity-purified Pc96 partially protected mice against challenge with the homologous parasite. To characterize Pc96 at a molecular level, monoclonal antibodies against this antigen were used to screen a genomic expression library in the vector lambda gt11. One clone was chosen for further study and a partial restriction map of the insert was determined. Southern and Northern analysis have been carried out and DNA sequencing of the entire coding region (2.8kb) has been completed. Although nucleic acid sequence homology with the gene encoding the RESA antigen of P. falciparum is minimal, the translated sequence has amino acid homology with this antigen and also shows an area of homology with erythrocyte band 3. A hydropathy plot of the predicted sequence suggests a very hydrophilic molecule with a hydrophobic region at the C-terminus. These studies may provide a basis for examining the biological role of this antigen as well as its importance as a vaccine candidate.

O 614 SURFACE LABELING OF EIMERIA TENELLA SPOROZOITES, M. Milhausen[1], C. Ko[1], K. Bafundo[2], M. Stiff[2]. Synergen, Boulder, CO 80301[1], Lilly Research Laboratories, Greenfield Laboratories, Greenfield, IA 46140[2]. Surface labeling of sporozoites from Eimeria tenella by two different procedures identified a limited number of major polypeptides. Labeling with Bolton-Hunter reagent detected the presence of four major polypeptides, migrating at 27, 24, 21 and 3 Kd on an unreduced SDS-PAGE. The 24 and 21 Kd species were shown to be the same or highly homologous polypeptides by immunoprecipitation with antisera to a clone isolated from a sporozoite cDNA expression library. Sequence analysis of this clone indicates that this polypeptide contains small stretches of a DNA motif common among the Eimerian genera. Western analysis of E. acervulina, E. brunetti and E. mitis indicates the presence of a highly cross-reactive homologue in each of these species. Chloramine T iodination of sporozoites revealed a single major labeled polypeptide migrating at 6 Kd on a reduced SDS-PAGE. This polypeptide is part of a larger protein which is synthesized as a 25 Kd protein and is processed several times into smaller components. Analysis of the synthesis of this protein throughout sporulation indicates that the protein is processed in part prior to sporocyste formation while additional processing occurs during a highly reactive homologue in other species. These data suggest a complex series of events may occur on the surface of the sporozoite from unique species specific genes which are proteolytically processed to polypeptides which may be common among the different species infecting avian hosts.

O 615 GLUCOSE UPTAKE BY TRYPANOSOMA BRUCEI, Teresita Munoz-Antonia*, Christian Tschudi*, Benjamin Spencer* and Elisabetta Ullu*+, MacArthur Center for Molecular Parasitology, *Department of Internal Medicine and +Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.
In Trypanosoma brucei, the requirement of glucose and its metabolism vary in different stages of the life cycle. It is not known whether the uptake of glucose in bloodstream and procyclic trypanosomes is by similar or different specific transport mechanisms. The specific uptake of glucose in bloodstream trypanosomes has been documented, but no attempts have been made to study glucose uptake in procyclics or to identify the molecule(s) involved in the transport. Using rapid centrifugation through a medium of defined density as a mean to separate free from intracellular glucose, we have measured glucose uptake in trypanosomes and shown that procyclic trypanosomes can uptake glucose in a specific, time- and dose-dependent manner. This uptake is inhibited by sulphydryl inhibitors and cytochalasin B. These compounds are known inhibitors of carrier mediated glucose uptake suggesting the presence of a glucose transporter in procyclic trypanosomes. These results are supported by the isolation of trypanosome cDNA clones that cross-hybridize with yeast and mammalian glucose transporter DNA probes.

O 616 A RECOMBINANT BABESIA BOVIS MEROZOITE SURFACE PROTEIN ELICITS ANTIBODY IN CATTLE THAT REACTS WITH LIVE MEROZOITES, David W. Reduker and Will L. Goff, USDA-ARS, Animal Disease Research Unit, Pullman, WA, Douglas P. Jasmer, Lance E. Perryman, William C. Davis and Travis C. McGuire, Dept. Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164. Ten monoclonal antibodies (MoAbs) were generated against five surface-exposed proteins (M_r 16 kDa, 44 kDa, 60 kDa, 225 kDa) on merozoites of Babesia bovis. These MoAbs were used to screen a λgt11 genomic library for the expression of recombinant surface proteins. A recombinant clone (λBo44-15) was identified that encoded a protein which bound a MoAb specific for native 44 kDa surface protein (Bo44). Southern blot hybridizations of bovine leukocyte DNA and merozoite DNA with radiolabeled Bo44-15 DNA confirmed the parasite-specificity of the cloned fragment. These assays indicated that the cloned sequence existed as multiple copies within either the parasite population or the genome of individual merozoites. Western blot analyses of λBo44-15 lysogen preparations demonstrated that recombinant protein production was IPTG-induced and the recombinant protein was a β-galactosidase fusion protein. Recombinant 44 kDa protein was purified by immunoaffinity chromatography and used to immunize cattle. Immune sera from these animals immunoprecipitated a metabolically-radiolabeled merozoite protein of 44 kDa and reacted by indirect immunofluorescence assay with the surface of live merozoites. Thus, these data indicated that antibody generated against recombinant Bo44-15 protein in cattle reacted with surface-exposed, native Bo44 protein.

O 617 TRAP, A MALARIA ANTIGEN WITH HOMOLOGY TO THROMBOSPONDIN, PROPERDIN AND CS PROTEIN. Kathryn J.H. Robson, Jennifer R.S. Hall, Gillian Cowan, K. Marsh, C.I. Newbold, R.A. Wells and D.J. Weatherall, MRC Molecular Haematology and Tropical Medicine Units, Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DU K. Takehara, I.M. Jones and D.H.L. Bishop. NERC Institute of Virology, Mansfield Road, Oxford.
We have recently described the cloning and sequencing of a new malaria gene TRAP (thrombospondin related anonymous protein) which bears striking homology to another malaria antigen, the circumsporozoite protein as well as to two host proteins thrombospondin and properdin. TRAP also shares with certain extracellular glycoproteins, including thrombospondin, the cell-recognition signal Arg-Gly-Asp(RGD), which has been shown to be crucial in the interaction of several extracellular glycoproteins with members of the integrin superfamily. Unlike the circumsporozoite protein TRAP is expressed during the erythrocytic stage of the parasite life-cycle. Current work involves the expression of TRAP using the baculovirus expression system, a selection system involving glutamine synthetase as well as β-galactosidase fusion proteins. Purification of TRAP from such systems will permit the generation of suitable immunological reagents to be used in experiments defining the function of this new antigen. Possible functions for TRAP include roles in cytoadherence based on the homology with thrombospondin, invasion due to the likeness to properdin or other cell-interactions involving parasite-host interactions. Other work on TRAP includes the study of restiction fragment length polymorphisms associated with the gene. Several polymorphisms exist, one occurs within the gene and the others involve flanking sequences.

O 618 COMPLEMENT RECEPTOR TYPE 3 BINDS TO AN ARG-GLY-ASP CONTAINING REGION OF LEISHMANIA GP63. David G. Russell and Samuel D. Wright, NYU Medical Center, 550 First Avenue, NY 10016, and Rockefeller University, NY 10021. Leishmania's survival is dependent on the promastigote entering and infecting the macrophage, a process known to be receptor-mediated. To identify the receptor for one of the ligands, gp63, the glycoprotein (J.Immunol.136.2613.1986) was reconstituted, together with phospholipids, onto the surface of a C18-derivatized silica bead. Attachment to human macrophages was dependent on the density of exposed gp63 (J.Exp.Med.168.279. 1988). Plating of macrophages onto antibodies against CR3 inhibited binding of gp63-beads. Synthetic peptides based on the RGD region of the primary structure of gp63 blocked binding of both gp63-beads and EC3bi to macrophages, as did intact gp63 made soluble by the removal of its phosphotidyl inositol membrane anchor. Similarly, other peptides known to inhibit the binding of C3bi also blocked gp63-bead attachment. Antibodies raised against the synthetic peptide from gp63 recognised the α-chain of human C3. Univalent F(Ab) fragments from this antibody also inhibited the attachment of gp63-beads to macrophages. These results demonstrate that the promastigote surface protein gp63 can bind directly to CR3 without the need for complement opsonization.

O 619 Leishmania LIPOPHOSPHOGLYCAN (LPG) BINDS TO THE CR3, LFA-1, p150,95 FAMILY OF ADHESION RECEPTORS. Patricia Talamas and David G. Russell. NYU Medical Center, 550 First Av. New York, NY 10016. Leishmania is an obligate intracellular parasite of the macrophage phagolysosome. Phagocytosis of leishmania promastigotes involves specific ligand-receptor interactions. The parasite molecules responsible for attachment to the host cell are a glycoprotein of 63 KDa (gp63) and the LPG. It was thought that LPG interacted with the mannose-fucose receptor, which is present on macrophages but not on monocytes. To identify the receptor for LPG, we tested the binding of LPG-beads to both monocytes and monocyte-derived macrophages. In both cases the beads bound strongly and trigger phagocytosis. The binding was dependent on the presence of cations and was abrogated by treatment of the macrophages with trypsin. In receptor "down-modulation" experiments, it was possible to correlate accessibility of the β-chain (CD18) of the CR3, LFA-1, p150,95 dimers as a requirement for LPG-mediated binding. The monoclonal antibody IB4, against CD18 inhibited binding about 50 to 70 %. Antibodies against the α-chain of CR3 (OKM1) or α-chain of LFA-1 (LeuM5) caused partial inhibition of LPG binding. A polyclonal antibody against the mannose-fucose receptor caused 30% inhibition. Interestingly, cells plated onto HSA + anti-HSA, or monoclonal antibody against FcR (3G8), increased the number of bound LPG-beads/cell.

O 620 **TRYPANOSOMA CRUZI MEMBRANE GLYCOPROTEINS INVOLVED IN ITS ADHESION TO MAMMALIAN CELLS, Fernando Villalta and Maria F. Lima, Division of Biomedical Sciences, Meharry Medical College, Nashville, TN 37208. Trypanosoma cruzi trypomastigotes must adhere to mammalian cells before they can penetrate them. We have analyzed the surface trypomastigote 83 kDa component that mediates the adhesion of trypomastigotes to heart myoblasts by 2-Dimensional PAGE. This adhesive component is not present on the cell surface of non invasive epimastigotes, but is expressed in trypomastigotes arising when epimastigotes differentiate into trypomastigotes. The bound trypomastigote 83 kDa component was dissociated from heart myoblasts, resolved by 2-Dimensional PAGE and analyzed by laser densitometry. Two 83 kDa glycoproteins of pI 6.4-6.7 and 6.9-7.1 were found to compose the 83 kDa band observed in one dimensional PAGE. The 83 kDa glycoprotein of pI 6.4-6.7 is the major component of this band. The major 83 kDa glycoprotein from highly infective trypomastigote clones associates more intensely with myoblasts than its counterpart from weakly infective trypomastigote clones. These results seem to indicate that the 83 kDa surface glycoprotein of T. cruzi trypomastigotes that binds to heart myoblasts is composed of a major glycoprotein of pI 6.4-6.7 and minor glycoprotein of pI 6.9-7.1. (Supported by NIH grant 1 R29 AI 25637-01A1 and AID grant DAN-5053-G-SS-8052-00).**

O 621 BINDING OF PLASMODIUM BERGHEI PROTEINS TO INSIDE-OUT VESICLES PREPARED FROM ERYTHROCYTE GHOSTS. M.F. Wiser*, A.C. Sartorelli and C.L. Patton. MacArthur Center for Molecular Parasitology, Yale U. School of Medicine, New Haven, CT. 06510. *Present Address: Department of Tropical Medicine, Tulane U. Medical Center, New Orleans, LA 70112

Two acidic phosphoproteins of *Plasmodium berghei* origin, with molecular masses of 65 kDa (Pb(em)65) and 46 kDa (Pb(em)46), are associated with the host erythrocyte membrane. Pb(em)65 partitions between the soluble and particulate phases upon host cell lysis, whereas Pb(em)46 is localized exclusively in the particulate fraction. Both proteins bind to inside-out vesicles (IOV) derived from mouse erythrocyte ghosts. The conditions of the reassociation reaction indicate that the binding is specific and that the proteins interact only with the cytoplasmic face of the erythrocyte membrane. Extraction of the IOV under alkaline conditions, before use in the binding assay, indicates that binding of the Pb(em)65 is only partially dependent on the presence of erythrocytic peripheral membrane proteins (e.g. ankyrin, bands 4.1, 4.2), whereas the binding of Pb(em)46 is absolutely dependent on these erythrocytic peripheral membrane proteins. Likewise, pretreatment of the IOV with papain only partially inhibits the binding of Pb(em)65. These results imply that Pb(em)65 interacts with both proteins and lipids of the erythrocyte and membrane. Furthermore, it appears that both Pb(em)65 and Pb(em)46 interact with the submembrane cytoskeleton of the erythrocyte. In summary, we have reconstituted some aspects of the host-parasite complex at the level of the erythrocyte membrane