MOLECULAR AND CELLULAR BIOLOGY OF HOST-PARASITE INTERACTIONS

Organizers: Nina M. Agabian and Harvey Eisen

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Plenary Sessions Page

Poster Sessions

Molecular Genetics of Protozoan Parasites

C 001 GENE EXPRESSION AND REPLACEMENT IN *LEISHMANIA*, Stephen M. Beverley, Angela Cruz, Cara M. Coburn, Jon LeBowitz, Diane McMahon-Pratt[2], Laura Rusche. Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston MA 02115, [2] Yale University Medical School, New Haven, CT.

We have developed methods for homologous gene replacement in *Leishmania major* (1,2). The targeting fragments in all cases consist of linear DNAs bearing *Leishmania* sequences flanking markers encoding either G418 or hygromycin B resistance (*neo* or *hyg*). The first studies with NEO constructs in the *dhfr-ts* locus showed that all transfected DNAs were found at the *dhfr-ts* gene. If high amounts of targeting fragment were used, insertion of multiple copies into the *dhfr-ts* locus were observed, as well as low levels of extra-chromosomal circular DNA. In contrast, reduced amounts of targeting fragment lead to replacement of the *dhfr-ts* coding region with *neo*. These *+/neo* lines were phenotypically normal.

Since *Leishmania* lacks an experimentally-demonstrable sexual cycle (3) and deletions are not available for most loci, we developed the method of double gene replacement to permit functional inactivation at any locus, using *hyg* as the second selectable marker. G418 and hygromycin B resistance conferred by *neo* and *hyg* are independent, permitting their use simultaneously or sequentially. Successive targeting of *+/neo* lines with *hyg* targeting fragments yielded *neo/hyg* lines that were thymidine auxotrophs and lacked *dhfr-ts* sequences; similar results were obtained with *+/hyg* lines and *neo* targeting fragments. In both cases, the efficiency of targeting in the second round was comparable to that seen in the first round. The method of double gene targeting should permit replacement of both copies of any locus, and conditional auxotrophs such as the *neo/hyg* lines may constitute useful strains for live parasite vaccination or the delivery of heterologous antigens.

Transcription in trypanosomatids is thought to occur in a polycistronic fashion. To help in our studies of this process, we have introduced a heterologous polymerase-promoter system into *Leishmania* promastigotes. The system that we have developed uses the T7 RNA polymerase and the 23 bp T7 promoter. Lines expressing two forms of T7 RNA polymerase have been developed, using pX-based expression vectors (1,4): one expressing native polymerase, and one expressing a modified polymerase containing an SV40 T antigen nuclear localization signal. Western blots show equals amounts of enzyme protein, but immunofluorescence studies show that the native polymerase is spread throughout the cytoplasm, whereas the T7nls polymerase is localized to the nucleus. This system functions well within *Leishmania*: insertion of the T7 promoter upstream of the splice acceptor sequence of reporter gene constructs expressing *E. coli* ß-galactosidase (5) results in 4-fold increased expression with native T7 polymerase, and more than 40-fold increases with the T7nls polymerase. The results obtained with this system in probing elements involved in polycistronic transcription will be discussed.

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C 002 MECHANISM OF HOMOLOGOUS RECOMBINATION IN LEISHMANIA ENRIETTII, Dyann F. Wirth, James F. Tobin, Avraham Laban and Maria Curroto de Lafaille, Harvard School of Public Health, Boston, Massachusetts.

Using our stable transfection system we have previously shown that promastigote forms of Leishmania enriettii contain the enzymatic machinery to mediate interplasmidic homologous recombination (1). We cotransfected two plasmids, containing nonoverlapping deletions of the bacterial chloramphenicol acetyl transferase (CAT) gene, into L. enriettii and demonstrated that a full-length CAT gene could be generated by homologous recombination. This work demonstrated that Leishmania enriettii contains the enzymatic machinery to mediate efficient interplasmidic homologous recombination and has allowed us to develop a method for cotransfection of two plasmids only one of which carries a selectable marker. In addition, we have devised a method for gene targeted insertional mutagenesis based on homologous recombination between exogenously added DNA and the targeted gene in the parasite chromosome. Analysis of such transfectants indicates that all of the insertion events are restricted to the homologous gene target. As little as 200 base pairs of sequence homology between the plasmid and the genome is required for integration. Nonhomologous recombination events are not detected. These results indicate that exogenous DNA sequences can be integrated into the L. enriettii genome provided that they are flanked by homologous DNA sequences. Finally, we have been able to delete one of the two tandem repeats of alpha tubulin genes using homologous recombination of flanking DNA sequences.

Cytokines in Host-Parasite Interactions

C 003 ANALYSIS OF CD4+ T CELL RECEPTOR USAGE IN MURINE LEISHMANIA MAJOR INFECTION. Richard M. Locksley, Steven L. Reiner, Zhi-en Wang, Bettie J. Holaday, Michael D. Sadick, and Farah Hatam. University of California San Francisco, San Francisco, CA.

Infection of BALB/c mice with Leishmania major leads to progressive dissemination of the parasite and fatal disease associated with expansion of CD4+ T cells that express mRNA for IL-4 and IL-10, consistent with a Th2 phenotype (1). A number of immunologic interventions, including sublethal irradiation or administration of anti-CD4+ or anti-IL-4 monoclonal antibodies, at the time of inoculation of the organism, results in control of Leishmania replication and establishment of immunity. In each case, such interventions are associated with expansion of CD4+ T cells that express mRNA for IL-2 and IFN-γ, consistent with a Th1 phenotype. Cell lines established from BALB/c mice with progressive infection or with established immunity are capable of transferring the respective phenotype to scid mice that are otherwise unable to contain L. major (2). A series of anti-T cell receptor Vβ monoclonal antibodies were used to analyze potential differences in T cell receptor usage in mice with progressive or controlled infection. Lymph node cells draining the sites of parasite infection were harvested and analyzed using FACS for Vβ expression. Among the antibodies screened, including anti-Vβ5, 6, 7, 8, 9, 11 and 13, there were no significant usage differences between the two groups of BALB/c mice in either the CD4+ or the CD8+ T cell populations. This analysis accounted only for 50% of the α/β+ T cells in the lymph nodes, however. In order to assess T cell receptor usage more completely, hybridomas were established using the appropriate lymph node cells and an α/β T cell receptor negative BW5147 thymoma cell line. Fusion cells were selected on the basis of α/β expression and production of Th1 or Th2 cytokines following stimulation. Eleven Th2 hybridomas and two Th2 parasite-specific clones were analyzed by reverse transcribing mRNA, PCR amplification using degenerate primers to screen all the murine Vβ T cell receptor family genes, and direct sequencing of the amplification products. Seven of the 13 used either Vβ4 or Vβ15. Of interest, of two Th1 clones analyzed, one used Vβ4 and the other Vβ15. These data suggest preferential usage of these two Vβ chains by parasite specific CD4+ cells in both healing mice and mice with progressive disease. (Supported by NIH AI30663)

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C 004 ANALYSIS OF THE ROLE OF SPECIFIC-CD4$^+$ T CELLS IN RESISTANCE AND SUSCEPTIBILITY OF MICE TO PRIMARY AND SECONDARY INFECTIONS WITH *LEISHMANIA MAJOR*, Jacques A. Louis[1], Fatima Conceiçao-Silva[1,3], Ingrid Müller[1], Ulrich Fruth[1] and Pedro Romero[2], World Health Organisation Immunology Research and Training Centre, Institute of Biochemistry, University of Lausanne, [2]Ludwig Institute for Cancer Research, Epalinges, Switzerland and [3]Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

Experimental evidence derived from the study of mice infected with *Leishmania major (L.major)* has demonstrated that acquired T cell responses are important in determining either resistance or susceptibility to infection with this parasite. It has been shown that CD4$^+$ T cells expressing a TH1 functional phenotype are preferentially expanding in lymphoid tissues of infected resistant mice. These cells exert an anti-*Leishmania* effector function through the lymphokines they secrete. Although interferon$_\gamma$ plays an important role in the elimination of *Leishmania* from infected hosts, experimental results suggest that resistance to infection requires the activity of other lymphokines. TH2 CD4$^+$ T cells have been shown to expand preferentially in susceptible mice infected with *L.major* and their effect on the disease process is at least partly due to the Interleukin 4 that they release.

Experimental evidence also indicates that not all TH1 like parasite-specific T cells are able to lead to the elimination of *L.major* from infected mice and it appears that their protective activity is related to their fine specificity. Recent results strongly suggest that T cells from the CD8$^+$ subset contribute to immunity against *L.major* under certain well defined experimental conditions. Eventhough the importance of CD8$^+$ T cells in controlling the course of a primary infection appears to be minimal, these cells are required for the establishment of resistance to reinfection.

Indeed, the protective role of CD8$^+$ T cells has been shown in resistant mice cured from a primary lesion as well as in susceptible mice rendered immune following immunological interventions such as: administration of anti-CD4 monoclonal antibodies at the beginning of infection, or anti-IL4 monoclonal antibodies during the course of a primary infection. In addition, significant numbers of CD8$^+$ T cells, able of transferring *L.major*-specific DTH responses to normal recipients,were found in lymphoid tissues of these mice. These findings indicate that *Leishmania*-specific CD8$^+$ T cells are induced during the course of infection.

Leishmania-specific CD8$^+$ T cell lines have been derived from the spleens of resistant mice that had recovered from a primary lesion. These lines displayed specific cytolytic activity against target cells sensitized with peptide digests from *L.major* promastigotes in a MHC class I restricted fashion. Strickingly, these cytolytic T lymphocytes (CTL) lines also significantly lysed syngeneic macrophages parasitized with *L.major*. This ability of MHC class I restricted CTL to recognize parasitized macrophages indicates that some antigenic peptides from *L.major*, which are confined to the phagolysozome compartment of their host cells, reach the class I MHC pathway of presentation (supported by the Swiss National Science Foundation and the World Bank/UNDP/WHO Special Programme for Research in Tropical Diseases).

C 005 CHAGAS'DISEASE: A PROBLEM OF CLASS REGULATION OF THE IMMUNE RESPONSE, Marcia Cury El Cheich[1], Antonio Coutinho[1], Doug Ross[2], Harvey Eisen[2], Mireille-Hontebeyrie-Joskowicz[1], and Paola Minoprio[1],[1] Department of Immunology, Pasteur Institute, Paris, France, [2] Fred Hutchinson Cancer Research Center, Seattle, WA.

T. cruzi parasite is known to induce a very high polyclonal activation of all classes of B and T lymphocytes. The patterns of antibody production and lymphokine secretion in the first two weeks of infection suggest an extensive CD4-dependence of polyclonal responses with a typical Th2-T cell participation. Thus, CD4-T cell depleted animals, as well as nude mice, while presenting increases in the number of circulating parasites, show a marked reduction of tissue inflammation. In addition, CD4 cell numbers are more elevated in sensitive strains of mice and, besides that, a CD4 cell line isolated from a chronically infected mouse was described as able to induce both polyclonal B cell activation and pathology to naive animals. We have observed that whenever the animals have their immune system compromised (lymphocyte depletions, anti-interleukine treatments "in vivo"), survival is prolonged even in the presence of higher parasitemia. These results though conflicting can be combined by the recently dichotomy between Th1 and Th2 CD4 cells which can mutually regulate their activities. We have recently proposed a potential role for CD5 B cells on the immune mechanisms induced by *T. cruzi* . These lymphocytes, particularly involved early in ontogeny, whose numbers are enhanced in autoimmune disorders, are a minor subset in adults. After *T. cruzi* infection, CD5 B cells are preferentially stimulated in parallel with DNT cells also known to be expanded in perinatal life. Those observations support our hypothesis of correlation between the expansion of these minor populations and the establishment of autoimmunity in late stages of disease. We have described that animals depleted on CD5 B cells - by lethal irradiation and adult bone marrow reconstitution- or Xid mice - bearing the Xid defect which leads to a block of CD5 B cell development-, when infected by *T. cruzi* display poor B cell responses (specific and non-specific antibody productions). These animals control parasitemia, do not show the typical wasting induced by infection and do not develop pathology. However, B cells from deficient mice conserve high levels of blast transformation in spite of no production of antibodies, suggesting that CD5 B cells could rather be implicated in the facilitation of B cell responses than in the production of the elicited antibodies. CD5 B lymphocytes could be controlling the repertoire of T and B cells by secreting lymphokines which could block Th1 activities or by enhancing MHC classe II molecules on B cells. Thus, it has been shown that IL-10, produced by Th2 are also produced by CD5 B cells and can mediate those activities. Indeed, by PCR amplification of RNA messages, or by *in situ* hybridization for the analysis of messager RNA at single cell level, we demonstrated that spleen cells from CD5 B cell deficient animals, in contrary to non deficient mice, show decreased IL-10 messages in the first two weeks of infection. More importantly, in the first 4 days, these animals show increased γIFN and IL-2 messages, Th1-lymphokines known to be suppressed in early phases of infection but the main mediators of parasite clearance. These results corroborate our claims that polyclonal activation accompanied by high levels of non-specific antibodies leading to an uneffective clearance of the parasite deal with a strong stimulation of one lymphocyte class (Th2) followed by an unbalanced response of the immune system. Therefore, the Th1 blocking observed in the early acute phase, if reverted could favor the prognosis of the disease.This work was supported by UNDP/World Bank/WHO-TDR. ID 910289

For review:
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Genomic Organization

C 006 MAPPING REGIONS OF THE MALARIA GENOME WITH YACS, David J. Kemp, Tony Triglia, Jenny Thompson, Debra Barnes, Freda Karamalis, Graham V. Brown, Karen P. Day and Alan F. Cowman, The Walter and Eliza Hall Institute of Medical Research, Melbourne Victoria 3050, Australia.

We have used the Yeast Artificial Chromosome (YAC) system to examine two regions of the *Plasmodium falciparum* genome. First, we have cloned large regions of a 110 kb amplicon bearing the pfmdr gene that is present in chloroquine resistant line B8. We have identified and sequenced the breakpoints by a novel method involving inverted PCR. We have shown that amplification of pfmdr1 has arisen as multiple independent events. Second, we have cloned a region of ~180 kb from near the right end of chromosome 9. This corresponds to a region that is often deleted during culture *in vitro* generating parasites that have lost the ability to express PfEMP1 and are non-cytoadherent. We are currently examining this cloned region in order to establish whether it encodes PfEMP1 or is a regulatory region.

C 007 REGULATION AND IMMUNOMODULATORY FUNCTION OF TH2 CYTOKINES IN PARASITIC INFECTION, Alan Sher[1], Ricardo Gazzinelli[1], Isabelle Oswald[1], Marika Kullberg[2], Jay Berzofsky[2], Herbert C. Morse[3], Edward Pearce[1], Tim Mosmann[4] and Stephanie L. James[1], [1]Immunology and Cell Biology Section, Laboratory of Parasitic Disease, National Institute of Allergy and Infectious Disease (NIAID), Bethesda, MD, 20892, [2]National Cancer Institute, Bethesda, [3]Laboratory of Immunopathology, NIAID, [4]Department of Immunology, University of Alberta, Edmonton, CAN.

The Th2 subset of CD4+ cells produces a series of cytokines (IL-4, IL-5, IL-6, IL-10) associated with B cell help as well as the allergic response. Recent data suggest that a number of these Th2 lymphokines may have important immunomodulatory functions inhibiting both the induction and expression of cell-mediated immunity (CMI). This relationship is exemplified in murine parasite infection models where the occurrence of Th2 responses often associates with a loss in CMI. While already documented in BALB/c mice with exacerbated *L. major* infection, the phenomenon is also evident in murine *schistosomiasis mansoni* where at the onset of egg-laying there is a concomitant burst in Th2 cytokine activity and suppression of Th1 responses to parasite antigen as well as mitogen. Moreover, mice during this period show decreased Th1 and augmented Th2 responses to injected myoglobin suggesting that this regulatory state extends to non-parasite antigens. The down regulation in Th1 response occuring after egg production is closely associated with the production of IL-10, and neutralizing anti-IL-10 antibodies up-regulate the suppressed parasite and myoglobin specific IFN-γ responses observed in vitro. In addition to down-regulating Th1 cytokine synthesis, IL-10 can directly suppress cell-mediated anti-parasite effector mechanisms inhibiting the activation of macrophages by IFN-γ for extracellular killing of schistosome larvae (schistosomula) as well as intracellular killing of *Toxoplasma gondii*. Both effects of IL-10 appear to be due to a suppression in nitric oxide production by the lymphokine activated macrophages. Moreover, in the case of schistosomulum targets, IL-10 synergizes with IL-4 and TGF-β in inhibiting IFN-γdependent killing. IL-10 and IL-4 induction are also associated with a retrovirus induced immunedeficiency (MAIDS) which can lead to re-activation of latent *Toxoplasma gondii* infection. Thus, the induction of host Th2 cytokines may promote parasite survival both by down-regulating Th1 cytokine production and inhibiting the activation of effector cells by the same mediators. Recent data (Clerici and Shearer, submitted) suggest that a similar imbalance may be induced by HIV infection leading both to viral progression and decreased resistance to opportunistic infections.

Host-Parasite Interactions: Cell Invasion

C 008 MALARIA MEROZOITE ADHESION PROTEINS AND ERYTHROCYTE INVASION, John W. Barnwell and Mary R. Galinski, Department of Medical & Molecular Parasitology, New York University Medical School, New York, New York 10010

The successful invasion of susceptible erythrocytes by malaria parasites is dependent on sequential interactions between specific components of the merozoite and the erythrocyte membrane. These molecular interactions initiate the process of parasite entry and determine host cell specificity. A number of merozoite adhesion proteins of Plasmodium falciparum and Plasmodium vivax and vivax -related parasites believed to be involved in the initial stages of invasion have been identified. The structures and the molecular basis for the interaction of these ligands with corresponding erythrocyte receptors are currently being investigated. Two adhesion proteins of P. falciparum merozoites that bind to susceptible erythrocytes have been identified, Pf MSP-1 and EBA-175. In correlation with the dependency of P. falciparum merozoite invasion on erythrocyte glycophorin both these proteins have been shown to bind to erythrocytes *via glycophorin. The binding in each instance is dependent on the* type and linkage of the sialic acid in the O-linked oligosaccharides of this erythrocyte membrane protein. Though the erythrocyte receptor specificity for both MSP-1 and EBA-175 appear to be similar, the two adhesion proteins are differentially localized; the former to the merozoite surface and the latter to the apical pole of the merozoite. This would suggest the two unrelated polypeptides have different functional roles during invasion. Several proteins of P. vivax merozoites have been shown to adhere specifically to certain primate erythrocytes. One of these is the approximately 140 kD Duffy Adhesion Protein (Pv DAP) that binds specifically to the erythrocyte Duffy blood group glycoprotein, an erythrocyte receptor known to be critical for invasion of human erythrocytes by P. knowlesi and P. vivax. The product(s) of the homologous P. knowlesi Duffy gene(s) have been localized to the merozoite micronemes prior to invasion. Two other P. vivax erythrocyte adhesion proteins (RBP-1 and RBP-2) of very high molecular weight (>250 kD) bind specifically to primate reticulocytes. P. vivax and P. cynomolgi invade exclusively reticulocytes and not normocytes. Southern blot analyses indicate the RBP-1 and RBP-2 genes are present only in these two malaria species and not in P. knowlesi, P. falciparum, or P. berghei. The two proteins are localized at the apical end of the merozoite and one (RBP-1) contains a single potential C-terminal transmembrane domain. Another P. vivax protein of about 210 kD also binds erythrocytes as does a 250 kD protein of P. knowlesi merozoites. The relationship of the Pv 210 and Pk 250 kD to each other or to other known merozoite antigens such as MSP-1 remains uncertain. However, in contrast to the binding of P. falciparum merozoite adhesion proteins and invasion, P. vivax and P. knowlesi invasion and erythrocyte adhesion protein binding to erythrocytes is not dependent on N- or O-linked oligosacharrides of the erythrocyte membrane receptor proteins. Future challenges will be to more fully characterize the molecular basis of the interactions of the merozoite adhesion proteins with their corresponding erythrocyte receptors and to determine the actual functional significance of these interactions in relation to events as they occur during invasion.

C 009 MEMBRANE PROTEINS INVOLVED IN THE ADHERENCE OF PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES TO THE ENDOTHELIUM, Irwin W. Sherman, Ian Crandall and Heidi Smith, Department of Biology, University of California, Riverside, California 92521.

The hallmark of Plasmodium falciparum infections is sequestration, that is, the attachment of erythrocytes infected with mature stage parasites to endothelial cells lining the post-capillary venules. In humans, the principal organs in which sequestration takes place are the heart, lung, kidney and liver. Sequestration in the brain microvessels -- a special pathology of P. falciparum infections called cerebral malaria -- may totally occlude blood flow and result in confusion, lethargy and deep coma. In this talk, we will review what is known about the molecular characteristics of the surface proteins -- that is, the red cell receptors and the endothelial cell (EC) ligands -- involved in sequestration. We will summarize recent work from our laboratory on the characterization of the adhesive proteins on the surface of the P. falciparum-infected red cell, and the ligands to which they bind on human brain endothelial cells (HBECs). Finally, we will discuss the possible role for "anti-adhesion therapy" in the management of severe malaria. Although many malarial antigens have been identified on the surface of, or in association with, the cytoskeleton of infected erythrocytes, only one protein, EMP 1, has been correlated with cytoadherence. The precise role of EMP 1 in sequestration has not been determined, principally because no monospecific polyclonal antiserum or monoclonal antibodies (Mabs) to it have been developed, nor has the gene for this protein been cloned. However, we have found that modified forms of the erythrocyte membrane protein, band 3, are directly involved in cytoadherence. Several murine monoclonal antibodies that specifically react with the surface of live infected red cells, and which recognize modified forms of band 3, were able to block cytoadherence in a dose-dependent fashion. These Mabs were used to map the epitope responsible for adherence, and chemically synthesized peptides conforming to the amino acid sequence of the epitope also blocked cytoadherence. The binding characteristics of amelanotic melanoma cells differed from both HBECs and HUVECs. Cytokine (TNF-α and IFN-γ)-stimulated HBECs were more adhesive than unstimulated HBECs or activated HUVECs. This increase in HBEC adherence appeared to be primarily due to upregulation of the surface ligand GPIV (= CD36), rather than ICAM-1. Based on our findings, it should be possible to synthesize small peptides and/or mimetics capable of adherence reversal, and these could be useful in the management of cerebral malaria.

Antigenic Polymorphisms

C 010 ANALYSIS OF A MAJOR SURFACE ANTIGEN GENE FAMILY OF *TRYPANOSOMA CRUZI*, Stuart Kahn[1], Wesley C. Van Voorhis[2], Trenton G. Colbert[3], Nicole A. Hoagland[4], James C. Wallace[4], and Harvey Eisen[4], [1]Department of Pediatrics, University of Washington, Seattle, WA 98195, [2]Department of Medicine, University of Washington, Seattle, [3]Department of Pathology, University of Washington, Seattle, [4]Fred Hutchinson Cancer Research Center, Seattle.

Trypanosoma cruzi is capable of infecting a wide variety of vertebrates and cell types. The parasite surface antigens exhibit extensive antigenic diversity. This diversity may play a role in the parasite's broad host range. During infection the parasite dysregulates the host immune response. Molecular mechanisms explaining these phenomena are poorly understood.

We have characterized a family of T. cruzi genes that code for a polymorphic set of 85-kD surface antigens. The initial characterization used DNA probes and antibodies to the C-terminal portion of several genes and antigens. The gene family includes >100 genes and pseudogenes, of which a minimum of nine are expressed. The gene family is expressed in the mammalian-stage only. A subset of the expressed genes are telomere-linked. Several subsets encode antigens that are simultaneously expressed on the parasite surface, and are released from the parasite surface into the surrounding milieu.

Further characterization of these genes revealed a region with significant homology to bacterial and viral sialidases. Antibodies to this region define a larger antigen family also of 85-kD. These antibodies immunoprecipitate sialidase activity. Anti-peptide antibodies define subsets of these antigen families. Some subsets includes sialidases, and others do not. It appears that this gene family may in part explain the diversity of the parasite surface. We suggest that this gene family may contribute to the parasite's ability to maintain a broad host range, and perturb the host immune response.

C 011 *EXPRESSION AND FUNCTION OF THE GP63 PROTEASE OF LEISHMANIA*, Robert McMaster, Linda Button, John Webb, Charlotte Morrison and Mary Macdonald, Department of Medical Genetics, University of British Columbia, Vancouver, B.C. V6T 1Z3.

The predominant surface protein of *Leishmania* promastigotes and amastigotes is a glycoprotein (GP63) of 63,000 M_r. GP63 is recognized by T cells in an infected mammalian host and thus may play an important role in immunity to *Leishmania*. Structural and functional studies have demonstrated that GP63 is a zinc metalloprotease although the enzymatic role of GP63 in the *Leishmania* life cycle remains an intriguing question. Recombinant GP63 has been produced by various eukaryotic expression systems including CHO, COS and baculovirus/insect cell lines. Recombinant GP63 was secreted as a latent protease and could be activated *in vitro* by an autocatalytic cleavage in the presence of organomercurial compounds and resulted in the removal of an amino terminal pro region. This activation mechanism is similar to proteases of the matrix metalloprotease family including interstitial collagenase and stromelysin. Based on this homology, GP63 may function as a matrix degrading enzyme which participates in the pathology of leishmaniasis.

To address the functional significance of GP63, homologous recombination is currently being used to generate strains of *Leishmania* with reduced or no GP63 expression.

GP63 is encoded by a family of tandemly linked genes which are highly conserved amongst diverse species of *Leishmania*. The regulation of GP63 gene expression is being studied by the characterization of genes encoding *Leishmania* nucleic acid binding proteins. The predicted protein sequence of one such gene encoding a DNA binding protein contains multiple zinc fingers homologous to those of retrovirus and human regulatory proteins. Studies indicate that the *Leishmania* nucleic acid binding protein recognizes specific DNA sequences located in the intergenic region of the tandem copies of GP63 genes. This protein may act as a trans acting factor involved with the regulation of GP63 gene expression.

C 012 SURFACE ANTIGENIC VARIATION IN GIARDIA LAMBLIA, Theodore E. Nash, National Institutes of Health, Bethesda, MD

There is a large degree of heterogeneity among Giardia lamblia isolates which can be broadly categorized into two definable types: those which are characteristic of the isolate and are generally a stable feature of the isolate (inherited traits) and those due to changes in surface antigens or antigenic variation. The latter can be demonstrated in clones and is a cause of heterogeneity within isolates and clones. Isolates have been compared using a variety of diverse techniques including RFLPs using a variety of probes, presence or absence of certain transcribed genes, characteristic rDNA nucleotide sequences, ability to express certain variant-specific surface proteins (VSPs) or epitopes, and the presence or absence of genes coding for specific VSPs. Surprisingly, with few disparities, isolates consistently fall within at least 3 groups. Group I encompasses those which resemble the prototype isolate WB. Group II shares some of the characteristics of WB while Group III shares few or none of the characteristics of WB and includes a number of unrelated isolates. A second source of heterogeneity is due to surface antigenic variation, a phenomenon exhibited by all Giardia lamblia. Each trophozoite expresses a single VSP which is spontaneously lost and replaced by another antigenically distinct VSP. The rate of appearance of VSPs is both isolate and epitope dependent and ranges from 1 for every 6-13 generations. VSPs vary in molecular weight from 35 kd to 200kd but share a number of common features. All VSPs including one expressed in an isolate from group III are cysteine-rich and have between 11-12% cysteine most commonly as cys-x-x-cys motifs. The carboxyl terminal regions appear well conserved but there are limited areas of identical amino acid sequences in other areas which explains why VSPs are antigenically distinct. Some VSPs have repetitive sequences and the epitope expressed is found in a number of isolates, but the VSPs differ in molecular weight. Most of the variability appears to be due to differences in the number of repeating units with conservation of the rest of the gene. In two VSPs studied the genes are present in two forms in the genome and in one instance was present in a tail-to-tail configuration separated by 3 kb. Clones eventually reexpress epitopes characteristic of its parental type but the epitopes expressed in some instances are restricted to certain isolates and fall into the same groups defined above. This indicates the same or similar VSPs can be expressed, lost and reexpressed. The repertoire size of one isolate was estimated using oligonucleotides to conserved or unique VSP sequences and found to range between 130-150 genes. These experiments suggested that there are 4 genes for every VSP so that the number of different VSPs is estimated between 30-40. The mechanism(s) responsible for antigenic variation are not known but the genes do not appear to be associated with the telomere. Antigenic variation occurs in experimentally infected humans, gerbils and mice. In humans, the initial VSP is lost and replaced by a series of variants by day 21 and humoral responses to the initial VSP are followed by responses to later appearing VSPs. Although the biological significance of VSPs are unknown, certain VSPs are more resistant to digestion by intestinal proteases suggesting a selective advantage of these VSPs for survival in the small intestine.

Towards Vaccination

C 013 PROTECTIVE ROLE OF THE 28 kDa SCHISTOSOME GLUTATHIONE S-TRANSFERASE, André Capron, Raymond J. Pierce, François Trottein, Delphine Grezel, and Chuan Bo Xu, Centre d'Immunologie et de Biologie Parasitaire, Unité Mixte INSERM U167-CNRS 624, Institut Pasteur, Lille, France.

Following the molecular cloning and expression of a protective 28 kDa protein of *Schistosoma mansoni* and its identification as a glutathione S-transferase, immunization experiments have been undertaken in several animal species. Together with a significant reduction in parasite burden, vaccination with Sm28 GST was recently shown significantly to reduce parasite fecundity and egg viability leading to a decrease in liver pathology. Whereas IgE antibodies were initially shown to be correlated with protective immunity, IgA antibodies to Sm28 GST have been identified as a major humoral factor affecting egg laying and viability. A significant correlation was found in a human population between anti-Sm28 IgA antibody production and acquisition of resistance to reinfection. IgA antibodies purified from human sera exert an inhibitory effect on the enzymatic function of Sm28 GST. The pertinence of this mechanism has been supported by the use of monoclonal antibodies. Recent experiments in which cattle were immunized with native *S. bovis* GST have confirmed this dual protective effect of vaccination. Based on the recent crystallisation of Sm28 GST and three dimensional analysis, together with the use of appropriate constructs, attempts are being made in order to optimize and direct the immune response through mucosal administration of the vaccine.

C 014 PROTECTIVE IMMUNITY WITH RECOMBINANT PEPTIDES OF S. MANSONI. Lorraine M. Amory, Claire P. Masterson, Timothy D. Tom, George H. Lowell*, and Mette Strand. The Johns Hopkins University, School of Medicine, Baltimore, MD 21205. *Walter Reed Army Institute of Research, Washington, D.C. 20307 Dr. Lowell contributed the meningococcal outer membrane protein.

We have previously reported the cloning of a cDNA clone, designated IrV-5, that encodes a peptide of 65 kDa. This recombinant polypeptide is recognized by sera of mice vaccinated with radiation-attenuated cercariae, but not by sera of chronically infected mice. Since it has been shown that immunoglobulins from sera of twice-vaccinated mice passively transfer resistance to naive mice, we tested the *immunoprophylactic potential* of the recombinant IrV-5 (rIrV-5) in mice. Results from several laboratories have shown that multivalent presentation is essential for the immunogenicity of an antigen. We used a system designed to hydrophobically complex synthetic peptides to multimeric protein preparations, or proteosomes, composed of meningococcal outer membrane proteins (OMP). *Initial vaccination trials using the rIrV-5* presented as a β-galactosidase fusion protein with OMP resulted in a 32% reduction in worm burden. To improve the immunoprophylactic potential, we then subcloned the IrV-5 cDNA into the pGEX vector to permit expression of rIrV-5 free of extraneous protein. Vaccination with the cleaved rIrV-5 proteosome resulted in a 75% reduction in worm burden. Mice were then immunized with varied doses of rIrV-5/OMP complexes prepared with varied ratios of rIrV-5 to OMP. The size of the rIrV-5/OMP complex was approximately 625,000 kDa irrespective of the rIrV-5:OMP ratio. Weight ratios of 0.5:1, 2:1 and 8:1 of rIrV-5 to OMP, respectively, were used with three vaccinations of 10 μg rIrV-5 or 10 μg OMP per mouse. These preparations elicited a high titered antibody response against rIrV-5 already after the second vaccination, and the titer remained essentially the same after the third vaccination. Three vaccinations yielded a 39% to 83% reduction in worm burden, whereas the level of protection achieved in mice vaccinated only twice was statistically insignificant. Furthermore, protection was not dependent on adjuvant, since BSA/OMP proteosomes failed to provide protection to vaccinated mice. In contrast, vaccination with rIrV-5 in the form of protein micelles resulted in a 48-62% reduction in worm burden. Two monoclonal antibodies generated from spleens of mice immunized with rIrV-5 and sera from mice vaccinated with rIrV-5 were used to immunoprecipitate an extract of surface-biotinylated schistosomula. Both the vaccine sera and the monoclonal antibodies immunoprecipitated a 200-kDa protein from this extract. Sera from mice twice-vaccinated with radiation-attenuated cercariae also precipitated a 200-kDa protein, whereas sera from chronically infected mice did not. Immunofluorescence studies confirmed the surface localization of the target antigen. Both the monoclonal antibodies and the sera from mice vaccinated with rIrV-5 bound to the surface of intact (diamidinophenylindole-negative) schistosomula. The results of protection studies performed in rats and baboons will also be reported.

Transcriptional Regulation

C 015 CONTROL OF EXPRESSION SITES FOR VARIANT-SPECIFIC SURFACE GLYCOPROTEIN GENES IN T.BRUCEI, Piet Borst, Joost C.B.M. Zomerdijk, Anneloor L.M.A. ten Asbroek, Janet Gommers-Ampt, Department of Molecular Biology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

African trypanosomes evade the immune response of their host by periodically changing their variant surface glycoprotein (VSG) coat. Each coat is encoded by a separate VSG gene. Expressed genes are in a telomeric expression site (ES). There are several sites in each trypanosome, but usually only one ES is active at a time. Coat switching either occurs by replacement of the expressed VSG gene, or by switching ES (see 1). Gene replacement is usually the result of a duplicative transposition event with the characteristics of a gene conversion. ES switching does not involve DNA rearrangements close to the promoter (2,3), but DNA rearrangements far upstream may accompany switching (3,4). The possibility that such rearrangements control the ES remains open. Other mechanisms invoked for ES control (see 5), include a novel form of DNA modification (6) involving PstI and PvuII sites and restricted to telomeric VSG genes that are not *transcribed. Using* ^{32}P-*postlabeling we have identified* and purified an unusual nucleotide pdJ in trypanosome DNA, that seems to account for the modified PstI and PvuII sites (6). pdJ differs from all known unusual eukaryotic deoxynucleotides; it is probably a pyrimidine nucleotide, but its structure is not yet known.

A remarkable feature of the transcription of VSG genes is its insensitivity to the RNA polymerase II *inhibitor α-amanitin (7). This has led* to the speculation that RNA polymerase I, normally only involved in the transcription of rRNA genes, also mediates the expression of these surface antigen genes. In higher eukaryotes, however, transcripts produced by RNA polymerase I were poor substrates for processing into mature mRNAs. In contrast, we have shown that the RNA polymerase I of T. brucei can mediate the efficient production of mRNAs for neomycin phosphotransferase. With the rRNA promoter region of T.brucei fused via a trans-splice acceptor segment to the CAT gene, transient CAT gene *expression in procyclic T.brucei* was as high as with CAT constructs driven by the parp-*a* (procyclin *a*) gene or VSG ES promoters. Stable trypanosome transformants were obtained by targeted insertion of a single neo' gene flanked by processing signals into a rDNA cluster downstream of a ribosomal promoter. These transformants produced neo-mRNAs carrying the 5'-capped spliced leader added via trans-splicing, and a poly(A) tail. The transcription of the neo'-gene was *α*-amanitin insensitive and started at the authentic RNA polymerase I initiation site. The neo-mRNA produced was functional as shown by the resistance of the transformed trypanosomes to high concentrations of the neomycin analogue G418 (2 mg per ml). Similar results were obtained with a transformant targeted into mini-chromosomes. It is therefore possible that VSG genes are transcribed by RNA polymerase I and that activation of an expression site requires the relocation of the site to the nucleolus.

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C 016 THE RNA POLYMERASES OF KINETOPLASTID PROTOZOA, Albert W.C.A. Cornelissen, Institute of Infectious Diseases and Immunology, State University Utrecht, Utrecht, The Netherlands.

Three classes of RNA polymerases (RNAPs) can be distinguished in eukaryotic cells by chromatographic separation. A principal method used to distinguish RNAPs in eukaryotes is the susceptibility of RNA synthesis to the cyclic octapeptide alpha-amanitin. This toxin inhibits RNA chain elongation and has a different affinity for the three classes of RNAPs. RNAP I, which transcribes rRNA genes, is insensitive. RNAP II, which takes care of the transcription of protein-coding genes, is very sensitive (I50% = < 0.05 ug/ml). RNAP III, which transcribed the small RNAs, has an intermediate sensitivity to the toxin (I50% = 10-25 ug/ml). In vitro transcription assays with Trypanosoma brucei nuclei (run-ons) showed an unusual property of the transcription units of the surface antigen genes of bloodstream- (VSG) and procyclic (PARP or procyclin) trypanosomes. Whereas transcription of protein-coding genes is more than 90% inhibited by 5ug alpha-amanitin per ml, VSG and PARP gene transcription is insensitive to 1000 ug alpha-amanitin. Although very likely, it has not yet been formally proven that the same, alpha-amanitin resistant RNAP is involved in these cases.

In order to obtain a possible explanation for the alpha-amanitin-resistant transcription in T.brucei, the trypanosomal RNAPs have recently been characterised by a molecular analysis of the genes encoding the largest subunits, transcription inhibition studies with different RNAP inhibitors, and by a biochemical analysis of the enzymes themselves (reviewed in (1)). This analysis was extended to a distantly related trypanosomatid species,Crithidia fasciculata (2,3), which at the transcriptional level shares most of its features with T.brucei. These data can be summarized as follows:
(i), in both species the normal set of eukaryotic RNAPs were identified by separating the RNAP I, II and III by activities by chromatography,
(ii), the largest subunits of RNAP I and III are encoded by single genes, but in T.brucei two genes encode the largest subunit of RNAP II; the second copy of RNAP II is only present is species exhibiting antigenic variation and may transcribe the VSG and PARP genes, and (iii), the carboxy terminal domain (CTD) of RNAP II is unique since it lacks the heptapeptide repeat (SPTSPSY) that is present in all other eukaryotes analysed to date.

To investigate the role of the second copy of RNAP II, we have generated class-specific antibodies. These antibodies were used as functional probes in run-on assays and in permeabilized cells (in collaboration with Dr. C. Tschudi, Yale University), however, none of the antibodies inhibited the RNAPs in either of these two in vitro systems. In an alternative approach, we constructed vectors for the targeted insertion of the bacterial neomycin phosphotransferase gene into the RNAP II loci, with the aim to specifically disrupt the RNAP II genes. This approach may allow us to determine the role of the two RNAP II genes.
We also started with the biochemical characterization of the aberrant CTD of T.brucei and C.fasciculata. The CTD of higher eukaryotes is essential for maximal transcriptional efficiency, and the CTD must be phosphorylated to permit a transition between initiation and elongation. We were able to demonstrate that a suramin-sensitive kinase specifically phosphorylates the CTD and one of the smaller subunits of RNAP II.

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C 017 GENETIC CONTROLS OF DIFFERENTIATION OF TRYPANOSOMA BRUCEI, Etienne Pays, Jean-Claude Jauniaux, David Jefferies, Pascale Paindavoine, Annette Pays, Sylvie Rolin, Patricia Tebabi, and Suzanne Van Assel, Department of Molecular Biology, Université Libre de Bruxelles, 67, rue des Chevaux, B1640 Rhode St Genèse, Belgium.

The life-cycle of Trypanosoma brucei involves the differentiation of the parasite into successive forms, the most prevalent being the long slender form in the bloodstream of the mammalian host, and the procyclic form in the midgut of the tse-tse fly. These forms are characterized by profound metabolic and phenotypic differences, which include the main surface proteins. The variant specific glycoprotein (VSG) and procyclin are the major components of the surface coat of bloodstream and procyclic forms, respectively.
We have analyzed the genetic controls underlying differential expression of surface antigens in T. brucei. The transcription promoters of both the VSG and procyclin genes have been mapped by taking advantage of the dual effect of UV irradiation on the parasite. We demonstrated that UV not only blocks RNA elongation, but also transiently inhibits RNA degradation. As a result of both effects, a specific accumulation of promoter-proximal transcripts occurs, which can be used to identify promoter regions. This technique has allowed us to characterize, not only promoters of stage-regulated genes, such as the VSG and procyclin, but also of constitutively expressed genes, such as actin. The nucleotide sequences of these promoters do not show significant homology. By run-on transcription analysis, we found that initiation of transcription occurs from both the VSG and procyclin promoters at both stages of the life-cycle, and that temperature-dependent attenuation of transcription is one of the control levels of differential gene expression. Using transient activity assays of the CAT reporter gene inserted into a plasmid construct electroporated into live trypanosomes, we have identified another apparent control at the level of RNA stability. In order to study the influence of the genomic environment on the control of gene expression during differentiation of the parasite, we have generated several stable trypanosome transformants. Plasmid constructs containing a gene coding for resistance to an antibiotic, placed downstream from the VSG promoter, were inserted at different genomic locations by homologous recombination. Stable transformants able to undergo the complete life-cycle could be obtained.
Several genes (ESAGs, for Expression Site-Associated Genes) have been found to be located between the VSG gene and its transcription promoter. By complementation of a yeast mutant, we have demonstrated that one of these genes, ESAG 4, encodes an adenylate cyclase. This gene belongs to a family of at least four members sharing a conserved 3'-terminal region most probably encoding the catalytic domain of adenylate cyclase. At least one of the ESAG 4-related genes (GRESAG 4.1) can also complement a yeast mutant for adenylate cyclase. Therefore, T. brucei contains at least two, but possibly more than four, distinct adenylate cyclases. While ESAG 4 is only expressed in bloodstream forms, the related genes are constitutively expressed in both bloodstream and procyclic forms. Immunocytochemistry experiments have revealed that the cyclases are transmembrane molecules located solely along the flagellum. The differential expression of ESAG 4 during the parasite life-cycle suggests that the function of the ESAG 4 adenylate cyclase may be linked to cell differentiation. Several lines of evidence point to a relationship between VSG release and activation of adenylate cyclase.

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Advances in Vector Study

C 018 MECHANISMS OF PARASITE RESISTANCE IN MOSQUITO VECTORS, Bruce M. Christensen, Department of Veterinary Science, University of Wisconsin, Madison, WI 53706.

Mosquito-borne parasites enter into highly complex relationships with both their vertebrate and invertebrate hosts, and successful completion of their life cycle is dependent on compatible associations within each these two very different environments. Although mosquitoes are required for the survival and transmission of certain filarioid nematodes, only certain vector species, or strains of a single species, are susceptible to infection and support the development of the parasite to the infective stage. Many strains and species are refractory (physiologically incompatible) or resistant (they destroy the parasite by internal immune reactions). Our research effort has been directed towards clarifying the mechanisms responsible for successful and/or unsuccessful mosquito-filarial worm associations by (1) assessing the biochemical and cellular events involved in melanotic encapuslation of parasites in resistant versus susceptible mosquitoes, and (2) evaluating the molecular mechanisms operating in defined strains of mosquitoes that are refractory to filarial worm infection. Studies on melanotic encapsulation reactions have focused on the development and utilization of highly sensitive and specific techniques to evaluate the enzymes and substrates involved in the production of protein-polyphenol substances required for parasite destruction. Radiometric and HPLC with electrochemical detection methods have been developed to assay monophenol and diphenol oxidase activity and catecholamine substrate changes in hemolymph samples, and have been used to assess the compartmentalization and relative roles these enzymes and substrates play in melanotic encapsulation within the hemolymph environment. These studies suggest that the effector mechanisms operating in mosquitoes is more complex than the simple hydroxylation of tyrosine to L-dopa and oxidation of dopa to dopquinone, with the subsequent production of melanin. Assessment by in vivo labeling of hemocyte and hemolymph plasma polypeptides has revealed unique and preferentially expressed molecules associated with melanotic encapsulation reactions. Likewise, several polypeptides are uniquely or preferentially expressed on the surface of hemocytes following immune activation and may be involved in signal recognition or effector events associated with melanotic encapsulation. To better facilitate the identification of genetic determinants of the immune response, and to identify the gene(s) and/or gene products responsible for susceptibility and/or refractoriness, we have begun construction of a RFLP map of Aedes aegypti, with an emphasis on saturation mapping linkage group I (the location of gene f^m that controls filarial worm susceptibility in this species), and have integrated our molecular markers with an existing mutant marker map for this species. In vivo labeling, SDS-PAGE, and two-dimensional gel electrophoresis with defined strains of A. aegypti also have been used to identify the expression of several unique polypeptides, induced by blood feeding, that correlate with filarial worm refractoriness. (Supported by NIH grants AI 19762 and AI 28781).

C 019 GENE EXPRESSION IN THE SALIVARY GLANDS OF MOSQUITOES, Anthony A. James[1], Genelle L. Grossman[2], Osvaldo Marinotti[3] and Kenneth R. Stark[1], [1]Department of Molecular Biology and Biochemistry, University of California, Irvine, CA, 92717, [2]Department of Tropical Public Health, Harvard School of Public Health, Boston, MA, 02115, [3]Departmento de Parasitologia, ICB, Universidade de Sao Paulo, C.P. 4365, 05508 Sao Paulo, S.P. Brasil.

The salivary glands of hematophagous mosquitoes have evolved to serve two feeding modes, sugar feeding, in which both males and females partake, and blood feeding, which is exclusive to females. A number of structural and functional properties correlate with the different feeding modes of the two sexes. The salivary glands of females are much larger and have lobes that are cytologically distinct from those found in males. Analysis of protein activities and gene expression in the female salivary glands indicates that the glands are functionally divided into two distinct regions. There are lobes that produce products involved in sugar feeding and appear to overlap the function of the male salivary glands, and there are lobes that produce female salivary gland-specific products presumably involved in blood feeding. We have shown that two genes presumably involved in sugar-feeding, *Mal I* and *Amy I*, encoding α-glucosidase and α-amylase respectively, are expressed in a restricted portion of the female salivary glands, the proximal lateral lobes, while they are expressed throughout the male salivary glands. Anti-hemostatic activities specific to female salivary glands including an apyrase are found in lobes distinct from those producing the carbohydrate metabolizing enzymes. Furthermore, female mosquitoes can regulate saliva secretion depending on the type of meal being taken.

The anti-hemostatic activities present in the female salivary glands have indicated the complexities of obtaining a blood meal from a vertebrate host. In addition to an apyrase that serves as an anti-platelet aggregating factor, we have demonstrated an anti-complement activity. Further studies on this activity are underway.

Parasites transmitted by mosquitoes may take advantage of the anti-hemostatic properties of the salivary gland in establishing infections in vertebrate hosts. It is clear that certain species of *Plasmodia* preferentially invade only those regions of the salivary gland used during blood feeding, thus optimizing their transmission to new hosts.

Post-Transcriptional Processing - The RNA Splicing Machinery

C 020 *TRANS*-SPLICING OF NEMATODE PRE-MESSENGER RNA, Timothy W. Nilsen, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

We have previously shown that a cell-free extract derived from developing embryos of the parasitic nematode *A. lumbricoides* catalyzes accurate and efficient *trans*-splicing of the *A. lumbricoides* SL RNA and a synthetic pre-mRNA containing a *trans*-splice acceptor site. Either endogenous SL RNA or SL RNA prepared by *in vitro* transcription can serve as the *trans*-splice donor. To function in *trans*-splicing synthetic SL RNA requires a functional Sm binding site but does not require cap hypermethylation.

We have used oligodeoxynucleotide directed RNaseH digestion to examine the U snRNA requirements for *cis*- and *trans*-splicing in the nematode extract. These experiments indicate that *trans*-splicing does not require the 5′ end of U1 snRNA but does require intact U2 and U4/U6 snRNPs; *cis*-splicing as expected requires an intact U1 snRNP. It has been shown by Bruzik and Steitz that utilization of SL RNA 5′ splice sites linked in *cis* to adenovirus 3′ splice site in HeLa cell extracts is independent of the 5′ end of U1 snRNA and that a determinant of U1 independence is the secondary structure (stem loop 1) involving the 5′ splice site.

To determine if this potential secondary structure contributed to U1 independent *trans*-splicing in the nematode cell free system, we used deletion and site directed mutation analyses. Surprisingly, these experiments indicate that neither the primary sequence nor the secondary structure of stem loop 1 of the nematode SL RNA is important for U1 independent *trans*-splicing. In the most extreme case, SL RNAs containing a two base exon participated in *trans*-splicing. These results suggested that the SL RNA could deliver completely heterologous exons via *trans*-splicing. To test this hypothesis, random fragments of pBR 322 were fused to the "intron" portion of the SL RNA. Transcripts containing pBR "exons" ranging in size from 26 to 246 bases were efficiently used as *trans*-splice donors. In each case *trans*-splicing required a functional Sm binding site.

Additional deletions and insertions were made within the "intron" portion of the SL RNA. These experiments indicated that the *trans*-splicing does not rely on a fixed distance between the splice donor site and the Sm binding site. To determine which sequences of the *Ascaris* SL RNA were relevant in *trans*-splicing, we performed extensive deletion and substitution mutagenesis as well as chemical modification interference analysis. These experiments indicated that the splice site itself as well as short region encompassing the Sm binding site and adjacent nucleotides were the only elements of the SL RNA critical for its function. To test the hypothesis that these features could confer the properties of an SL RNA to a heterologous RNA we constructed modified U1 snRNAs which contained a functional splice site and the SL RNA Sm binding region. Remarkably, these chimeric constructs functioned in *trans*-splicing with an efficiency comparable to that of the wild type SL RNA.

C 021 mRNA BIOGENESIS IN AFRICAN TRYPANOSOMES, Elisabetta Ullu[1,2], Keith Matthews[1], and Christian Tschudi[1], Yale MacArthur Center for Molecular Parasitology, [1]Department of Internal Medicine and [2]Cell Biology, Yale School of Medicine, New Haven, CT 06510.

In trypanosomes protein coding genes are organized in polycistronic transcription units. In order to produce mature monocistronic mRNA molecules, the mRNA coding regions embedded within polycistronic pre-mRNAs are excised via RNA processing reactions. Specifically, trans-splicing of the spliced leader sequence generates the 5' end and cleavage and polyadenylation the 3' end of mRNA molecules. Previously, we had established a permeable cell system which is competent for transcription and trans-splicing. We have now shown that 3' end formation of the alpha and beta tubulin mRNA is also efficiently carried out in permeable trypanosomes. We are using this system to characterize the interplay between trans-splicing and polyadenylation and their requirements. In addition, we have assembled a number of artificial monocistronic and dicistronic reporter genes which differ either at their 3' end or in the intergenic region. These constructs are being used to identify cis-acting elements required for polyadenylation and to evaluate the potential role of polyadenylation in modulating the output of mature mRNA.

Host-Parasite Interactions: Parasite Antigens

C 022 CELL SURFACE PROTEASES OF MALARIA PARASITES, Catherine Braun Breton, Anne-Catherine Fricaud, Jean-Christophe Barale, Thierry Blisnick and Gordon Langsley, Institut Pasteur, Paris France

The role of a chymotrypsin-like serine protease in red blood cell invasion by malaria merozoites has been established. We have identified a glycosyl-phosphatidylinositol anchored serine protease with properties consistent with it being the enzyme required for erythrocyte invasion: specific inhibitors lead to a marked decrease in infectivity and, importantly, without affecting merozoite attachment. The serine protease activity is developmentally regulated and released from invading merozoites.

To further characterise this enzyme, we have performed *in vitro* assays of red blood cell invasion. The effects of specific inhibitors and of pretreatment of the red blood cells with the purified enzyme have been investigated. Our progress in the biochemical characterisation and gene cloning of this serine protease will be reported.

As part of our characterisation of parasite-specific proteases present at the surface of the infected red blood cell, we have shown that the host plasminogen activator urokinase (uPA) binds specifically to the surface of the infected erythrocyte. The kinetics and specificity of this binding as well as its role in the parasite life cycle are being investigated.

C 023 T-DAF: A DEVELOPMENTALLY REGULATED COMPLEMENT INHIBITOR EXPRESSED BY TRYPOMASTIGOTES OF *T. cruzi* Denise V. Tambourgi[1], Gary B. Ogden[2], Keith A. Joiner[2], Lee F. Hall[2], Alan Sher[3], Steve Heath[3], Wilmar Dias da Silva[4], Thereza L. Kipnis[1]. [1]Universidade de São Paulo, São Paulo, Brazil, [2]Yale University School of Medicine, New Haven, [3]National Institutes of Health, Bethesda, [4]Instituto Butantan, São Paulo, Brazil.

Resistance to Complement (C) mediated killing is an important acquisition for the infective forms of Trypanosoma cruzi. While epimastigotes (Epi) are C susceptible the metacyclic trypomastigote (MT) which develops within the vector, the bloodstream trypomastigotes (BT) and the tissue culture derived trypomastigotes (TCT) are C resistant. We have shown that BT, TCT and MT produce molecules which interfere with the efficient assembly of the C_3 convertases of both classical and alternative pathways. This C_3 convertase inhibitory activity was detected in supernatants recovered from parasites incubated for 10 minutes at 45°C or for 4 hours at 37°C. Further analysis showed that these supernatants accelerated the decay of the C_3 convertases but did not have factor H-like cofactor activity for factor I-mediated cleavage of C_{3b} to iC_{3b}. Therefore, the trypomastigote supernatants contained functional activity analogous to human decay-accelerating factor, a complement regulatory membrane surface glycoprotein, which limits complement activation and lysis on cells by homologous complement components. For this reason we called the components present in the trypomastigote supernatants with complement inhibitory activity as T-DAF. This C_3 convertase inhibitory activity present in the trypomastigote supernatants was co-purified from an FPLC chromatofocusing column with an 87-93 kDa band and this component could be destroyed by papain and retained on concanavalin A-Sepharose column, suggesting that the trypomastigote supernatant molecule with functional decay-accelerating activity is a glycoprotein.

The 87-93 kDa components were recognized in immunoprecipitation assays or ELISA by sera from patients chronically infected with T. cruzi. Lytic activity for tissue culture trypomastigotes in sera from infected mice was also associated with antibody reactivity against this protein.

Monospecific polyclonal serum raised against T-DAF molecule was capable of partially blocking its biologic activity and this serum was used to screen a lambda gt11 cDNA expression library prepared from mRNA of metacyclic trypomastigotes of T. cruzi (Miranda 88 strain). Thirteen positive clones were isolated, from which five were purified to homogeneity and subsequently shown to contain the same partial cDNA insert by DNA sequence analysis. Substantial homology (40-45%) was found between the nucleotide sequence of the positive cDNA insert and the sequences established for human DAF and mouse factor H.

Southern analysis revealed that the cloned cDNA insert hybridized to one or two genomic DNA digestion fragments of T. cruzi (Y strain) and by Northern blot it was shown that the gene correspondent to the cloned sequence is expressed in trypomastigote forms but not in epimastigotes, suggesting that its expression is developmentally regulated.

The recombinant fusion protein was able to inhibit the C_3 convertase assembly in a dose dependent fashion and antibodies raised against it were able of promoting complement mediated lysis of trypomastigotes.

C 024 *PLASMODIUM FALCIPARUM*: THE BIOLOGY AND CHEMISTRY OF HEMOZOIN, Andrew F.G. Slater, Picower Institute for Medical Research, 350 Community Drive, Manhasset, New York 11030.

The malaria parasite *Plasmodium falciparum* invades and grows within the red blood cells of its human host for a significant portion of its life cycle. The developing trophozoite ingests hemoglobin from the cytosol of its host cell, digesting it in a food vacuole to provide amino acids for its continued growth. Proteolysis of hemoglobin releases heme, which if soluble can damage biological membranes and inhibit a variety of enzymes. As malaria parasites lack heme oxygenase, they are unable to cleave heme into an open chain tetrapyrrole, and it is not excreted from the cell. Instead heme is detoxified by conversion into a microcrystalline substance called hemozoin or malaria pigment, which accumulates within the food vacuole of the trophozoite concomitant with hemoglobin breakdown. Hemozoin is released along with the merozoites when the infected red cell bursts and is scavenged by macrophages. The pigment is insoluble under physiologic conditions, and remains undegraded within tissue macrophages of the host for an extended period of time.

We have purified hemozoin from *P.falciparum* trophozoites, and have used a variety of chemical methods to determine its structure. Although several proteins, of which human globin is the most significant, intimately associate with hemozoin during its isolation, all of these proteins can be removed without a detectable change in the chemistry of the constituent hemes. No modifications to either the porphyrin macrocycle or the side-chains of heme were detected. The hemozoin crystal was found to consist of an unusual polymer of hemes linked between the central ferric ion of one heme and a carboxylate side-group oxygen of another. This linkage protects the carboxylic acid functional group from solvation, and allows two or more monomers to polymerise into hemozoin. Preliminary data on the space groups of the hemozoin crystal have recently been obtained.

Hemozoin does not form spontaneously from either free heme or hemoglobin under physiological conditions, and the biochemistry of its formation has not been previously studied. We have identified an enzymatic activity, which we call heme polymerase, in extracts of malaria trophozoites that catalyses the formation of hemozoin. The enzyme works optimally between pH 5.0 and 6.0, which covers the estimated pH of malaria food vacuoles. We have also found that the activity is inhibited by quinoline-containing antimalarial drugs such as chloroquine and quinine. The possible significance of this in the antimalarial action of these drugs will be discussed.

Mitochondria, Glycosomes and Hydrogenosomes

C 025 BIOGENESIS OF HYDROGENOSOMES OF TRICHOMONAS VAGINALIS, Patricia J. Johnson, Carol J. Lahti, Peter J. Bradley and Christine E. d'Oliveira, Department of Microbiology & Immunology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

Trichomonas vaginalis is an anaerobic, flagellated protist which resides in the urogenital tract of its human host. Trichomonads lack mitochondria and instead contain an unusual organelle involved in carbohydrate catabolism: the hydrogenosome. Hydrogenosomes are the site of fermentative oxidation of pyruvate, coupled to ATP production via substrate level phosphorylation. The organelle is surrounded by two membranes and appears to contain no genetic material. Hydrogenosomes are proposed to have arisen either through the conversion of mitochondria or via endosymbiosis with an anaerobic bacterium. To gain a better understanding of the origin and biogenesis of the hydrogenosome, we have characterized two hydrogenosomal proteins, as well as the genes which encode these proteins. Our studies show that hydrogenosomal proteins are synthesized on free polyribosomes, and are thus post-translationally translocated into the organelle. These data also indicate that hydrogenosomes multiply by fission, as opposed to budding from the rough endoplasmic reticulum. The two hydrogenosomal proteins we have examined, ferredoxin and the beta subunit of succinyl CoA synthetase, appear to be synthesized as larger precursors that contain an eight or nine amino acid NH_2-terminal extension, respectively. These apparent leader sequences are absent from the mature proteins found in hydrogenosomes. The biochemical properties of the NH_2-terminal extensions are strikingly similar to one another, and to mitochondrial presequences. These data support the hypotheses that the specificity of protein translocation into hydrogenosomes is conferred by a short NH_2-terminal signal sequence and that the mechanisms underlying translocation are similar to that of mitochondria. To address these hypotheses we are currently establishing an in vitro import assay using purified hydrogenosomes, as well as studying the protease responsible for the cleavage of hydrogenosomal proteins.

C 026 A SUBSET OF THEILERIA PARVA MITOCHONDRIAL PROTEINS IS ENCODED BY A LINEAR 7.1 KBP DNA MOLECULE, Vishvanath Nene, Elke Gobright and Alladin Kairo, International Laboratory for Research on Animal Diseases, P. O. Box 30709, Nairobi, Kenya.

Theileria parva is a tick transmitted protozoan parasite which causes a lymphoproliferative and usually fatal disease in cattle. The parasite is characterised by two intracellular stages, a schizont stage in lymphocytes and a piroplasm stage in erythrocytes. Analysis of total piroplasm T. parva genomic DNA on agarose gels reveals the presence of an "extra-chromosomal" genetic element that migrates at 7.1 kbp on agarose gels. This element is a double stranded DNA molecule which hybridises only to itself in Southern blot analysis of total genomic DNA suggesting that the element contains unique DNA sequences. The restriction map of the element is consistent with a linear rather than a circular structure and the linearity has been confirmed by demonstrating that the element is susceptible to Bal31 digestion. About 6 kbp of the element has been cloned and sequenced. The A+T composition is about 70% and the ends of the cloned DNA contain inverted and direct sequence repeats. The DNA sequence encodes potential genes for cytochrome b, polypeptide I and a polypeptide III-like subunit of cytochrome c oxidase and there are a few short, dispersed stretches of sequences which have a high degree of similarity with rRNA sequences. Northern blot analysis shows the presence of major transcripts of 1.3, 1.1, 0.25 and 0.07 kilobases in size. One near full length cytb cDNA clone has been sequenced and other cDNA clones are being characterised. The finding of genes coding for mitochondrial proteins on the 7.1 kbp DNA element suggests that this DNA is mitochondrial in origin and these features of the T. parva DNA are reminiscent of the mitochondrial genome of Chlamydomonas reinhardtii, a unicellular green alga. Five other species of Theileria and two species of Babesia contain a similar size molecule to the T. parva element. There are simple and distinct restriction fragment length polymorphisms between the elements which makes the 7.1 kbp DNA a useful diagnostic probe for single and mixed parasite infections. A multiple tandemly repeated unit of 6 kbp has been described in Plasmodium species with each unit potentially encoding similar genes to those found on the T. parva element. It appears that parasitic organisms in the phylum Apicomplexa which have been tested so far contain an unusual type of DNA encoding a limited number of genes normally found on mitochondrial DNA. There is, however, plasticity in the structure and copy number of the DNA and the organisation of the genes encoded.

C 027 STRUCTURE FUNCTION RELATIONSHIPS OF THE GLYCOSOME, Frederik R. Opperdoes, International Institute of Cellular and Molecular Pathology, B-1200 Brussels, Belgium

Glycosomes, microbody-like organelles present in all Kinetoplastida studied (i.e. *Trypanosoma, Leishmania, Crithidia, Phytomonas* and *Trypanoplasma*), are unique to these organisms in that they are the only members of the peroxisome family that contain the early enzymes of glycolysis, two enzymes of glycerol metabolism, enzymes of CO_2 fixation and adenylate kinase. Catalase may or may not be present. In the African trypanosome *T.brucei* glycolysis is characterized by an extremely high carbon flux through the glycosome. This high flux is facilitated by the high concentrations of catalytic subunits and their respective substrates inside the glycosome, which may be close to 1 mM. To facilitate interaction of the densely packed glygolytic enzymes with their negatively charged substrates, glycosomal enzymes have almost invariably incorporated additional positively charged amino acids in unique peptide insertions and/or in N- or C-terminal extensions. As a consequence these enzymes carry a high net positive charge and have isoelectric points between 8 and 10. In those organisms where the glycolytic flux plays a less important role in overall energy metabolism (e.g. *T.cruzi, Crithidia fasciculata, Leishmania mexicana* and *Trypanoplasma borelli*) the glycolytic enzymes analyzed all have the unique amino-acid insertions and extensions similar to *T.brucei*, but lack the positively charged amino acids. Several, but not all, of the glycolytic enzymes of *T.brucei* carry at their C-terminus a so-called peroxisome targetting signal (PTS). When present in a *T.brucei* enzyme a similar PTS is also found in the homologous sequences of the other kinetoplastida analyzed.

C 028 EVIDENCE FOR THE MECHANISM OF RNA EDITING, Larry Simpson, Agda M. Simpson and Beat Blum, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024.

A new model for RNA editing has been proposed. In this model two successive transesterifications bring about the transfer of U residues from the 3' tail of a gRNA to an editing site. The function of the terminal uridylyl transferase is to add U residues back to the 3' tail of the gRNA. The gRNA in this model functions both as a guide sequence and as a donor of U's for editing. Evidence for this model involves the demonstration by PCR of the chimeric gRNA/mRNA molecules which are predicted transient intermediates in this process. In vitro editing experiments are also being performed to test this hypothesis. In the first experimental system, the label is supplied as [^{32}P]UTP and the localization of label in synthetic cytochrome b mRNA is followed by RNase H digestion with specific oligomers. In the second experimental system, the label is supplied as 3'-end labeled MURF3 gRNA and the incorporation of label into synthetic MURF3 mRNA is followed by gel electrophoresis.

C 029 TARGETING THE GLYCOSOME, Jürg M. Sommer[1], Qi-Lin Cheng[1], Gilbert-A. Keller[2], and C. C. Wang[1], [1]University of California, San Francisco, CA 94143, and [2]Genentech Inc., South San Francisco, CA 94080

The compartmentalization of glycolytic enzymes into specialized organelles, the glycosomes, allows the bloodstream form of *Trypanosoma brucei* to rely solely on glycolysis for its energy production. The biogenesis of glycosomes in these parasites has been intensively studied as a potential target for chemotherapy. An *in vitro* assay of 3-phosphoglycerate kinase (PGK) import into *T. brucei* glycosomes was established. It demonstrated specific uptake of denatured glycosomal enzyme (gPGK), but was unable to take up the highly homologous cytoplasmic enzyme (cPGK). The gPGK taken up by intact glycosomes is resistant to 3M urea and proteinase K, and thus may represent protein import across the membrane structure without proteolytic modification. More recently, we adapted the stable transformation of *T. brucei* for *in vivo* analysis of glycosomal protein import. A peroxisomal protein, firefly luciferase, was expressed in the procyclic form of *T. brucei* by polycistronic transcription of the luciferase and the neomycin phosphotransferase (*neo*) gene utilizing a promoter for one of the procyclic acidic repetitive protein (PARP) genes of *T. brucei*. Stable transformants were obtained by selection for G418-resistant cells following integration of the linearized DNA construct into a tubulin intergenic locus via homologous recombination. Cloned cell lines carried between 6 and 30 copies of the luciferase gene distributed over 3 insertion sites within the tubulin gene cluster, resulting in a relatively high level of luciferase expression. Cell fractionation and immunoelectron microscopy indicate that luciferase accumulates inside the glycosomes of *T. brucei*. Replacement of the C-terminal leucine in luciferase with valine by site-directed mutagenesis resulted in substantial accumulation of the mutant protein in the cytoplasm. This conservation of a protein import mechanism in peroxisomes and glycosomes supports the previously proposed evolutionary relationship between these two microbodies.

C 030 PLASTID DNA IN THE APICOMPLEXA ? Iain Wilson[1], Malcolm Gardner[1], Don Williamson[1], and Jean Feagin[2].[1] National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K. [2] Seattle Biomedical Research Institute, Seattle, WA 98109-1651.

Malaria parasites carry two extrachromosomal DNA molecules with organellar characteristics. One is a 6kb reiterated sequence, arranged in linear tandem repeats. This molecule specifies at least two mitochondrial proteins as well as blocks of sequence allocated to fragmented small and large subunit rRNA genes and is presumed to represent the mitochondrial genome. A second DNA molecule, the so-called "35 kb circle", codes for two sets of full length rRNA genes arranged in a novel form of inverted repeat reminiscent of chloroplast genomes. Of the protein-encoding genes so far identified on the 35 kb circle, the most striking are rpoB and rpoC, juxtaposed genes encoding subunits of a eubacterial type of RNA polymerase. Such genes have never been reported on mitochondrial genomes but are a feature of chloroplasts. The organization of the malarial rpoB gene resembles that of chloroplasts rather than E.coli. Subcellular fractionation studies indicate that the 6 kb element and the 35 kb circle occupy different cellular compartments. Molecular phylogenetic studies of nuclear rRNA genes [1,2] place the origins of malaria and dinoflagellates within a relatively short evolutionary distance of each other. We propose that the progenitor of malaria, and several other Apicomplexan parasites, was photosynthetic and that a plastid remnant of unknown but essential function has been retained to the present day. This organelle has yet to be identified at the subcellular level but the "spherical body" is a likely candidate in malaria parasites.

1.Gajadhar,A.A. et al. 1991 Mol Biochem Parasit. 45, 147.
2.Nanney,D.L. et al. 1991 J Mol Evol 32,316.

Opportunism

C 031 MOLECULAR AND CELLULAR BIOLOGY OF *CRYPTOSPORIDIUM PARVUM*, Carolyn Petersen[1], Richard G. Nelson[1,2], Lisa Gooze[1], Jiri Gut[1], Patricia S. Doyle[1], Joseph H. Crabb[3] and James H. Leech[1]. [1]Parasitology Laboratory, San Francisco General Hospital, Departments of [1]Medicine and [2]Pharmaceutical Chemistry, University of California at San Francisco, San Francisco, California and [3]ImmuCell, Portland, Maine.

Cryptosporidium parvum, an Apicomplexan parasite of intestinal epithelial cells, is a common cause of diarrheal disease in children and residents of the tropics and is a cause of life-threatening malnutrition and dehydration in 15% of persons with AIDS in the US. In spite of the prevalence and morbidity of *Cryptosporidium* infection in persons with AIDS, little is known about the biology or biochemistry of the organism and no effective treatment regimens are available. We have prepared monoclonal antibodies (MAbs) to sporozoites and intracellular parasites, obtained hyperimmune bovine colostral IgG, constructed genomic DNA libraries and developed an *in vitro* model of sporozoite invasion and development to begin to investigate aspects of the biology and molecular biology of *Cryptosporidium* which may allow the development of effective immunotherapy and/or chemotherapy.

Antibodies to pellicle and apical complex proteins of invasive stages of other Apicomplexan parasites such as *Toxoplasma, Plasmodium* and *Eimeria* have been shown to protect against parasite challenge. Hyperimmune bovine colostrum (HBC) raised to *Cryptosporidium* oocysts/sporozoites has been reported to cure AIDS patients and protect calves against *Cryptosporidium* challenge. We have begun to characterize 12 major antigens identified by protective HBC IgG on Western blot of oocyst/sporozoite proteins. In an alternate strategy, we have identified 10 candidate sporozoite pellicle and apical complex proteins based on IFA pattern with monospecific reagents. Five of these candidates were identified with antibodies eluted from lambda gt 11 DNA expression library clones (REAs) and five were identified with MAbs to sporozoites. One antigen, a >500 kD glycoprotein of sporozoites and merozoites, is highly immunogenic. It is the major antigen detected by HBC IgG and is the the target antigen of one REA, 3 sporozoite-reactive MAbs and 2 intracellular stage-reactive MAbs.

We have cloned and sequenced the gene encoding *Cryptosporidium* dihydrofolate reductase (DHFR), a target enzyme of anti-folate antibiotics, and intend to express and kinetically analyze the encoded protein to determine whether the refractoriness of *Cryptosporidium* infection to treatment with available antifolates is due to unique structural features of the enzyme. *Cryptosporidium* DHFR, like that of other protozoans, is encoded by a bifunctional gene which also encodes thymidylate synthase (TS). Cloning of the gene was made possible by PCR amplification of a segment of the TS gene using degenerate oligonucleotide primers based on highly conserved TS sequences.

Cryptosporidium has been thought to be related to *Eimeria* and *Isospora* on the basis of ultrastructural observations and similarity of host cell. 18S ribosomal analysis, however, suggests that the organism is phylogenetically more closely related to *Sarcocystis* and *Theileria*. Electrophoretic karyotype analysis indicates that *Cryptosporidium* has at least 5 and perhaps 7-9 chromosomes which range in size from 0.9 mb to 1.5 mb. The genome is estimated to be 10-15 mb, much smaller than that reported for other Apicomplexans including *Plasmodium, Eimeria* and *Toxoplasma*.

C 032 HIGH FREQUENCY SWITCHING IN CANDIDA ALBICANS, David R. Soll, Brian Morrow, Jan Schmid, Srikantha Thyagarajan, Department of Biology, University of Iowa, Iowa City, Iowa, 52242.

Almost all tested strains of Candida albicans and related species are capable of switching at high frequency and reversibly between a number of different general phenotypes which can be distinguished by colony morphology and in some cases cellular morphology and physiology. There are a number of different switching systems, defined by colony phenotypes in the switching repertoires, but it appears that each strain possesses a single switching system. The best studied switching system is the white-opaque transition in strain WO-1, obtained from the blood of a bone marrow transplant patient. It has been demonstrated that the white-opaque transition affects virtually every putative virulence trait of C. albicans, involves differential gene expression, and affects every aspect of cellular architecture including the genesis of unique pimple structures in the opaque cell wall. We have cloned genes which are differentially expressed both in the opaque and white switch phenotype. One opaque-specific DNA encodes a pepsinogen-like protein with strong homology to pepsinogens of Penicillium, monkey, pig and human. However, it is unusual in that it contains only one, rather than two, aspartyl-containing active site, similar to primitive pepsinogens encoded by retroviruses. Although it is clear that switching affects many traits, is frequent and reversible, and occurs at sites of commensal carriage and disease, it is not clear what role it plays in pathogenesis. Recently, a study was performed in which strains were isolated from different body locations of the same healthy individuals, fingerprinted with the moderated repetitive DNA probe Ca3, and the Southern blot hybridization patterns analyzed for similarity with the Dendron software package. Simultaneous isolates from the oral cavity and vaginal canal in all of 11 cases were nonidentical. In 7 cases, the pairs were genetically unrelated, but in 4 cases genetically similar but nonidentical suggesting that they had evolved from the same progenitor. It is argued from these indirect observations that one role of switching is to provide a strain with the initial phenotypic variability in a colonizing population for adaptation to different environmental niches in the same host, and that persistence of these different switch phenotypes is alternative niches reduces mixing, resulting in isolation and genetic divergence.

Molecular Biology - I

C 100 Trypanosome Mitochondrial Ribosomal RNAs Contain Poly-Uridine Tails, Brian K. Adler, Michael E. Harris, Karen I. Bertrand and Stephen L. Hajduk, Depts. of Medicine and Biochemistry, University of Alabama at Birmingham, AL 35294

The mitochondrial ribosomal RNAs of *Trypanosoma brucei* are unusual due to their small size, organization of their genes, and developmental regulation. Despite the small size of the 9S (640 nt) and 12S (1230 nt) rRNAs, they resemble the 16S and 23S rRNAs of *E. coli* which suggests that the trypanosome ribosomes may contain other RNA components or have unusual structures. The steady state levels of the mitochondrial rRNAs in *T. brucei* correlate with the developmental regulation of mitochondrial biogenesis. There is a 30 fold supression of the mitochondrial rRNAs in the blood stream compared to insect form of the trypanosome, and this is probably regulated at the level of RNA stability. We now show another unusual aspect of the mitochondrial 9S and 12S ribosomal RNAs, modification of the 3′ termini by uridine addition. Studies in our laboratory on the editing of mitochondrial mRNAs indicated that the mitochondrial rRNAs were also modified by the addition of uridines. Metabolic labeling studies using intact mitochondria and [α-^{32}P]-UTP, in the absence of transcription, demonstrated the post-transcriptional timing of the event. T_1 ribonuclease comparison analyses of [^{32}P]-pCp 3′ end labeled and [α-^{32}P]-UTP metabolically labeled rRNAs, along with direct RNA sequencing of the 3′ termini, identified the site of uridine addition, and revealed the creation of an oligo(U) tail for both rRNAs. 12S and 9S rRNAs hybrid selected from total cell RNA exhibited the same modification demonstrating the presence of this processing *in vivo*. Moreover, only 3′ poly(U) tailed 9S and 12S rRNAs were detected in total cell and mitochondrial RNA which suggests that they are the most abundant and probable mature forms. The 12S and 9S rRNA oligo(U) tails differed significantly from each other with the 12S having a heterogeneous tail of 2-17 uridines, and the 9S a precise length of 11 uridines. The mechanism of formation and the function of the rRNA poly(U) tails remain to be determined.

C 101 PRODUCTION OF A CHROMOSOME-SPECIFIC LIBRARY FOR *Leishmania major*. James W. Ajioka, Anita C. Skinner and Jenefer M. Blackwell, Department of Medicine, University of Cambridge Clinical School, Addenbrooke's Hospital, Cambridge UK CB2 2QQ

Although the chromosomes of *Leishmania* are readily resolvable by pulse field gel electrophoresis (PFGE), very few marker genes have as yet been assigned to specific chromosomes. As part of a study of the molecular determinants of parasite virulence, we have generated 48 clones of *L. major*, 10 of which have been analysed in detail for their infectivity *in vivo* and *in vitro* and for their expression of major molecular determinants (LPG, GP63 and HSP70) of virulence. PFGE revealed significant karyotypic variations in clones of differing virulence, but without a good physical/genetic map it has proved difficult to trace the associated chromosomal rearrangements. We have therefore initiated a programme of research ultimately aimed at production of chromosome-specific libraries for all chromosomes. Our first libraries have been prepared from chromosomes to which GP63 and HSP70 have already been assigned, and taking as our source DNA the standard laboratory LV39 strain. Chromosome specific DNA was excised from the gel following PFGE in low melt agarose, and the gel segments equilibrated in buffer/Sau3A prior to addition of $MgCl_2$ and incubation for 30 min. The partially digested DNA was eluted from the gel and recovered by phenol/chloroform extraction and alcohol precipitation, prior to loading onto a standard agarose gel for size fractionation of 12-20 kb insert DNA. DNA recovered following size fractionation was ligated into the BAMHI site of EMBL3 and grown on *E. coli* strain P2393. Chromosome specific libraries are being analysed for representation of known single copy/tandemly repeated genes (GP63, HSP70) and methods developed to assess the number, type and distribution of repetitive sequences.

C 102 THE ANATOMY OF THE SL RNA GENE ARRAY IN THE TRYPANOSOMATID PROTOZOA: PRESENCE OF SITE-SPECIFIC RETROTRANSPOSONS (SITEPOSONS) AND GENETIC LINKAGE TO 5S rRNA GENES, Serap Aksoy and Merceditas S. Villanueva, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 066510

Retrotransposon sequences with a ubiquitous genomic distribution have been reported in *Trypanosoma brucei*. Unlike these elements, SLACS (also called MAE) in the African trypanosome *T.brucei*, CRE1 in the mosquito trypanosomatid, *C.fasciculata*, CZAR in the New World trypanosome *T.cruzi* and HER1 in Herpetomonas spp. are associated with only one locus, the SL RNA gene cluster(s). All four elements interrupt their host sequences in a site specific fashion, ie. they are inserted between nucleotides 11 and 12 of the SL RNA gene coding sequence. DNA sequence analysis has shown that they have the hallmarks of non-LTR retrotransposons: extensive target site duplications at their insertion, 3'poly(A) tails and one or two long Open Reading Frames with motifs homologous to retroviral GAG and POL sequences. Although each element has distinct primary sequence, all three share extensive amino acid homology in the conserved POL domains. Their overall structural organization and the highly conserved domains within their ORFs, suggest that they represent an evolutionarily related family of elements.

Analysis of the SL array in a different New World trypanosomatid, *T.rangeli*, has shown that the SL RNA genes are organized within the same repeat unit sequences with the 5S rRNA genes. This alternating SL and 5S arrangement is also conserved in the African trypanosome *T.vivax* and in Herpetomonas. In all cases, the orientation of transcription for both genes is in the same direction. Genetic linkage for the SL and 5S loci had been noted in nematodes but not been previously observed in Trypanosomatids.

C 103 PRODUCTION OF NITRITE BY Bcg^s AND Bcg^r MACROPHAGE LINES. Luis F. Barrera, Emil Skamene and Danuta Radzioch.

Department of Experimental Medicine, McGill Centre for the Study of Host Resistance, Montreal General Hospital Research Institute, Montreal, Quebec, Canada H3G 1A4.

It had been demonstrated that different mouse strains exhibit a differential ability to control the growth of several species of mycobacteria including *M. bovis* BCG. It has been shown that this property is regulated by the expression of the *Bcg* gene.

The Nitric oxide ($NO^{\cdot}$), a metabolite derived from L-arginine, has been associated with the capacity of macrophages to control the growth of several intracellular parasites including mycobacteria.

We compare the NO_2 production in bone marrow-derived macrophage lines B10S and B10R established from congenic strains of mice susceptible (B10.A, *Bcg*^{r}) or resistant (B10A.*Bcg*^{r}) to *M.bovis* BCG, respectively.

Our results indicate that the B10R macrophage lines produce twice as much NO_2 than the B10S macrophage lines in response to BCG infection, stimulation with rIFN-γ, or a combination of both stimuli. The levels of NO_2 production were higher when IFN-γ acted as a first signal as compared to BCG.

Thus, the differential capacity of B10R and B10S macrophage to produce reactive nitrogen intermediates (RNI) might be closely associated with their ability to inhibit mycobacterial growth.

C 104 *ANTIGENIC VARIATION IN PLASMODIUM FALCIPARUM*, B-A. Biggs[1], L. Goozé[2], K. Wycherley[1], W. Wollish[2], B. Southwell[1], J. H. Leech [2], R.F. Anders[1], G. V. Brown[1]. [1] Immunoparasitology Unit, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia. [2] The Medical Service, San Francisco General Hospital and Department of Medicine, University of California at San Francisco, California 94710, USA.

Antigenic variation of surface molecules expressed by certain infectious organisms is a major factor in evasion of the host immune response. There has been no definitive demonstration of this phenomenon in the malaria parasite, *Plasmodium falciparum*. In this study, cloned parasites were examined serologically and biochemically for the expression of antigens at the erythrocyte surface. A cloned line of *P. falciparum* gave rise to progeny that expressed antigenically distinct forms of an erythrocyte surface antigen but were otherwise identical. This demonstrates that antigenic differences on the surface of *P. falciparum*-infected erythrocytes can arise by antigenic variation of clonal parasite populations. The antigenic differences were shown to result from antigenic variation of the parasite-encoded protein, the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). The emergence of *P. falciparum* variants appears to be influenced by certain host-dependent factors.

C 105 PROCESSING OF THE PLASMODIUM FALCIPARUM MEROZOITE SURFACE PROTEIN 1: IDENTIFICATION OF A 33 KILODALTON PRODUCT OF A SECONDARY PROTEOLYTIC PROCESSING EVENT AND PARTIAL CHARACTERISATION OF THE PROTEASE INVOLVED, Michael J. Blackman, Irene T. Ling, Hilton Whittle and Anthony A. Holder, National Institute for Medical Research, Mill Hill, London, U.K.

We have previously shown that only a single 19kd fragment of the Plasmodium falciparum merozoite surface protein 1 (MSP1) is carried with an invading merozoite into the infected red cell. This fragment ($MSP1_{19}$) is derived from the C-terminal, membrane-bound end of a major product, $MSP1_{42}$, of the primary stage of MSP1 proteolytic processing. Using a monoclonal antibody mapped to an epitope within the N-terminal region of $MSP1_{42}$, we have shown that a soluble 33kd polypeptide ($MSP1_{33}$) corresponding to the N-terminal region of $MSP1_{42}$ is shed into culture supernatants during merozoite release and erythrocyte invasion. These observations provide further evidence that the secondary processing of $MSP1_{42}$ involves a highly specific proteolytic activity. Amino acid sequencing of the N-terminus of the $MSP1_{19}$ species has determined the site specifity of this protease, and suggests that the cleavage site is conserved between the different allelic types of MSP1. The pattern of sensitivity of the protease to various inhibitors has provided further information as to its location and mechanism of action.

C 106 DEVELOPMENT OF GENETICS FOR MAPPING OF DRUG RESISTANCE TARGETS AND VIRULENCE DETERMINANTS IN *TOXOPLASMA*. John C. Boothroyd, L. David Sibley, and Elmer Pfefferkorn*. Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA, *Microbiology Department, Dartmouth Medical School, Hanover, NH

We have recently constructed a combined physical and genetic map for Toxoplasma to facilitate the application of classical genetics in addressing aspects of intracellular parasitism. In all, 70 random DNA probes were mapped by hybridization to gel-separated chromosomes and by segregation of RFLP markers among 19 recombinant progeny following a genetic cross between P and C strains. The resulting map provides 1 marker spaced approximately every 1.0 Mb of the genome that corresponds to a resolution of 5-10 cM in genetic distance. Using this map, preliminary genetic linkage between specific RFLP markers and unknown genes of interest is feasible to within 100-500 kb, as demonstrated for mutants resistant to AraA (linkage group V) or sinefungin (linkage group IX). This strategy is currently being applied to mapping the targets of several promising new drugs for treatment of toxoplasmosis and for investigating mutants with altered invasion (Inv^-) or adherence (Adh^-) phenotypes.

To explore the natural variation of genetic polymorphisms from a wide range of Toxoplasma strains, four RFLP markers that are unlinked were developed for a PCR-based approach to RFLP mapping. In all, 27 independent strains were analyzed from three groups; animal infections, human congenital infections, and human AIDS infections. Strains from these three groups exhibit a similar level of genetic divergence and polymorphism and do not show marked host or geographical correlations with particular genotypes. However, a striking relatedness was evident for the 10 strains that exhibit high virulence in mice ($LD_{100} < 10$); all have highly similar genotypes at the four genetic loci. This correlation was 100% for one of the four markers located on chromosome VIII where all 10 virulent strains had the identical genotype whereas the 17 nonvirulent strains ($LD_{100} > 10^3$) all had a second allele at this locus. The 10 virulent strains were originally isolated from both animal infections and human infections that had resulted in ocular, congenital, or AIDS CNS toxoplasmosis. Additionally, these strains were isolated from widely separate areas in the USA, Brazil and France suggesting that clonal propagation contributed to this pattern of distribution. Based on the preliminary correlation of specific RFLP markers with virulence, it should be feasible to map the molecular basis of virulence by classical genetic analyses.

C 107 USE OF A GENE PROBE TO DETERMINE THE EFFECT OF HOST AND PARASITE GENOTYPE ON THE SPECIFICITY OF CRISIS IMMUNITY IN RODENT MALARIA, BOURNE, T., JARRA, W., SNOUNOU, G., BROWN, K.N., Division of Parasitology, NIMR, Mill Hill, London, England.

During a primary malaria infection in the non-immune host the reduction of parasite numbers from dangerously high levels to lower, less critical levels is crucial if the host is to survive. This reduction in parasitaemia is called crisis. Since crisis is central to protective immunity and because naturally acquired malaria infections frequently consist of a mixture of parasite strains and species, it is necessary to understand the immune mechanisms involved in crisis and establish their specificity.

Using the CBA/Ca inbred mouse model and cloned parasite lines, parasite clearance during crisis and thus the effector mechanism(s) involved have been shown to be predominantly species- and strain-specific.

A gene probe PCsv4.1, developed in this laboratory, can be used to differentiate parasite species, sub-species and strains, even when they are present concurrently. Thus the probe can be used to analyse the dynamics of individual parasite strains in mixed infections, providing a basis for the investigation of immunity to mixed infections.

Evidence is presented which indicates that the marked specificity of the mechanism(s) responsible for the clearance of parasites at crisis is independent of both host (including H-2) and parasite genotype.

C 108 Cysteine proteinases from *Leishmania major* as targets for chemotherapy. Jacques Bouvier*† and Judy Sakanari*. *Department of Pathology, UCSF School of Medicine, San Francisco, CA, and †Insititut de Biochimie, Université de Lausanne, CH-1066 Epalinges, Switzerland.
There has been increasing interest in proteolytic enzymes because of their critical roles in the pathogenesis in several parasitic diseases. As targets for the design of new nontoxic drugs to treat these diseases, they offer an alternative to vaccination. Proteinase inhibitors represent an important class of drugs already in use for a variety of diseases. Cysteine proteases of *Leishmania major* are important for the metabolism of the organisms, and the specific inhibition of these proteinases using "designed" inhibitors will arrest the development of the parasites. The cysteine proteinases of *L. major*, promastigotes were extracted in 2% Triton X-114, and were purifed by several steps of chromatography. Purified proteinases hydrolyze the synthetic chromogenic peptide substrates Z-phe-arg-*p*-nitroanilide and Z-arg-arg-*p*-NA, and are inhibited by two nontoxic inhibitors of cysteine proteinases, Z-phe-ala-fluoromethylketone and Z-phe-arg-fluoromethylketone. Preliminary results indicate that these inhibitors arrest growth of the parasites within infected macrophages *in vitro*.
One of the genes has already been isolated from an *L. major* genomic library by screening with a *Trypanosoma cruzi* cysteine proteinase gene fragment. Sequence analysis shows that the *L. major* gene shares great similarity to other members of the cysteine proteinase family. (supported by UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases no. L30/181/82).

C 109 IDENTIFICATION OF TWO *LEISHMANIA* H REGION GENES USING MULTICOPY EXPRESSION VECTORS, Heather L. Callahan, William Roberts*, Petrie Rainey*, and Stephen M. Beverley, Dept. Biol. Chem. and Mol. Pharm., Harvard Medical School and *Dept. Lab. Med., Yale New Haven Medical School
Transfection of vectors which are multicopy and thereby over-express passenger DNA has been used in many systems to identify genes of interest. Amplification of the *Leishmania* H region, a 48 kb circular, extra-chromosomal DNA, has been correlated with resistance to many drugs including methotrexate and Na arsenite. Using our multicopy *Leishmania* transfection vectors we identified two H region genes, one which confers metal resistance but not MTX resistance, and one which confers MTX resistance but not metal resistance. The MTX resistance gene has been mapped to a 6 kb piece of DNA and is currently being sequenced. The metal resistance gene is an *L. major* P-glycoprotein homologue (*lmpgpA*) which confers resistance *in vitro* to both arsenite and trivalent antimony (SbIII), but not pentavalent antimony. Although *Leishmaniasis* is treated with pentavalent antimonials, researchers have postulated that trivalent antimonials are the active species. If this hypothesis is correct, *pgpA* may play a role in clinical resistance. The mechanism of drug resistance is also unclear. Unlike cells with classical MDR phenotypes, *lmpgpA* transfectants are not resistant to vinblastine, nor is their resistance reversible by verapamil. Resistant transfectants do show a two-fold decrease in steady-state drug accumulation (SbIII) when compared to control cell lines. We are currently testing whether the 10-fold increase in drug resistance may be linked to changes in efflux, since other P-glycoproteins have been shown to function as efflux pumps.

C 110 ISOLATION AND CHARACTERIZATION OF THE BETA-TUBULIN GENE OF *BABESIA BOVIS*. Rosanne E. Casu, CSIRO, Division of Tropical Animal Production, Indooroopilly, Qld, 4068. AUSTRALIA.
Microtubules are essential, multifunctional, subcellular components found in all eukaryotes. They are involved in chromosome segregation (mitotic spindle), cell structure, motility (cilia and flagellae) and transport phenomena. Microtubules are formed by the polymerization of dimers of non-identical alpha- and beta- tubulin subunits, which have regions of homology. Tubulins also show a high degree of evolutionary conservation. In *Babesia bovis*, an intraerythrocytic protozoan parasite of cattle, microtubules form the third layer of the pellicle of the pyroform body. A microtubule layer is also present in the parasite in its invertebrate stages, radiating from a polar ring situated in the blunt end. From studies on other parasitic organisms it could be supposed that interference with the structure of the microtubules would grossly alter the ability of the organism to survive.

The most conserved region of tubulins in general is an area near the carboxy terminus. A short synthetic oligonucleotide corresponding to this region was used to screen a *Babesia bovis* cDNA lambda gt10 library, yielding two independent clones. The insert from the longer clone was subcloned and both ends sequenced. This insert codes for about 80% of the *B. bovis* beta-tubulin gene, encompassing the central portion of the gene. It was subsequently used to screen a *B. bovis* lambda gt11 library, resulting in a full-length clone which has been fully sequenced. Five different independent clones have also been isolated from a *B. bovis* genomic DNA EMBL3 library using this probe. The most suitable of these has been subcloned and sequenced, and its structure analyzed.

C 111 CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED cDNAs OF *Eimeria bovis* SPOROZOITES AND MEROZOITES. Timothy G. Clark, Mitchell Abrahamsen, Patrice L. Mascolo, C.A. Speer, and Michael White, Veterinary Molecular Biology, Montana State University, Bozeman, MT 59717

Eimeria bovis is an obligate intracellular parasite that causes coccidiosis in cattle. The developmental pathway for *E. bovis* includes five distinct morphological cell types: the sporont of unsporulated oocysts, the invasive stages, sporozoites and merozoites, and the sexual stages, macro and microgametocytes. To initiate studies on the developmental regulation of gene expression in the invasive stages, we prepared λgt11 cDNA libraries from mRNA isolated from sporozoites or merozoites. Differential screening of the merozoite and sporozoite cDNA libraries were performed using probes generated from both sporozoite and merozoite mRNAs. From these screens, 67 independent clones and over 250 clones were obtained that appeared to be more highly expressed in merozoites or sporozoites, repsectively. Several clones or each type have been characterized and used as probes for Northern hybridizations to confirm the differential expression. The genetic structure and pattern of mRNA expression during parasite development for several merozoite and sporozoite specific cDNAs will be presented.

C 112 CHARACTERIZATION OF THE GENE FOR GP72, AN INSECT STAGE-SPECIFIC ANTIGEN OF *TRYPANOSOMA CRUZI*, Robin Cooper, Jill A. Inverso, Amelia Ribeiro de Jesus, Martha Espinosa, Nadia Nogueira, and George A.M. Cross, Laboratory of Molecular Parasitology, The Rockefeller University, New York, NY 10021.

A 72 kD glycoprotein, GP72, is an important developmentally-regulated surface antigen of *Trypanosoma cruzi*. Studies using the monoclonal antibody WIC 29.26, which recognizes a carbohydrate epitope, indicate that GP72 may be expressed only in epimastigotes and metacyclic trypomastigotes. The function of GP72 remains unclear. However it may play a role in the control of cellular differentiation through interaction with insect gut lectins. Purified GP72 was partially sequenced and oligonucleotides derived from these data were used to amplify and identify a partial cDNA. A genomic clone encoding the entire glycoprotein was then isolated and sequenced. The primary translation product is 581 amino acids long with a molecular weight of 62,600. The protein has a characteristic N-terminal signal sequence and a hydrophobic C-terminal domain, confirming that it is likely to be membrane localized. The protein sequence has five potential N-linked glycosylation sites and a large number of serines and threonines that are potential sites of O-glycosylation, including a region of 43 amino acids composed of 42% serine or threonine and 40% proline, a common theme in O-glycosylated regions of other glycoproteins. These findings are consistent with previous studies which characterized the composition of GP72. No significant homologies were found in searches of DNA and protein databases. A 4,000 nt transcript was found in epimastigote polyA+ RNA. The GP72 gene has been expressed in bacteria and polyclonal antibodies are being raised against the purified fusion protein. In addition we are attempting to delete the two GP72 alleles in live parasites by transfecting with gene replacement constructs. These studies will allow new insight into the regulation, structure and function of GP72.

C 113 *ENTAMOEBA HISTOLYTICA* PATHOGEN-SPECIFIC GENOMIC AND ASSOCIATED cDNA FRAGMENTS HYBRIDISE TO POLYMORPHIC SEQUENCES. Jorge A. Cruz-Reyes and John P. Ackers, Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London WC1E 7HT,UK.

Recent data by different groups indicate that pathogenic and non-pathogenic *E. histolytica* are genotypically different (eg. 1-3). The genome structure and regulation of gene expression in pathogenic *E. histolytica* have been poorly studied, although the organisation of an extrachromosomally located ribosomal circle in this organism has been well documented (4). Here, we present preliminary results on the organisation of a 3kb pathogen-specific genomic sequence IE-1. This DNA sequence hybridises to a 0.7kb RNA transcript. Southern blots of digested DNA from the pathogenic strain HM1:IMSS probed with either a coding fragment of IE-1, a 0.5kb distant non-coding fragment, or a cDNA clone with homology to IE-1, suggest that the IE-1 locus is related to an abundant highly polymorphic group of sequences. Nucleotide sequence analysis of IE-1 and of independent cDNA clones is being performed. Initial results of pulse field CHEF analysis indicate that the entire fragment, IE-1, hybridises to different bands with sizes ranging from 0.2Mb to 2Mb.

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C 114 SEQUENCE ANALYSIS OF *Tritrichomonas foetus* rDNA UNIT TO DESIGN OLIGONUCLEOTIDE PROBES TO DETECT PARASITES IN PREPUTIAL SAMPLES, John B. Dame[1], Debopam Chakrabarti[1,2], and Charles A. Yowell[1], [1]Department of Infectious Diseases, College of Veterinary Medicine, [2]Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL 32611-0633

The flagellated, anaerobic protozoan, *Tritrichomonas foetus*, is the causative agent of bovine trichomoniasis, a contagious sexually transmitted disease characterized by infertility, abortion, and pyometra. The ribosomal RNA (rRNA) unit (rDNA unit) of the protozoan parasite has been cloned and analyzed for the production of species-specific oligonucleotide probes to detect the organism in bovine preputial samples. The rDNA unit occurs in the genome in a tandem head to tail array of 12 units with a single unit length of 6 kb. A primary transcript of 5.8 kb was detected by northern blot analysis. Sequence analysis combined with comparisons to conserved 5' and 3' boundary sequences of the 16S-like, small subunit rRNA (16S-like rRNA) and 5.8S rRNA, defined coding regions of 1571 bp and 159 bp, respectively. The short *T. foetus* 16S-like rRNA sequence is comparable in size to those from two other amitochondrial protists and phylogenetic analyses are underway. Despite its small size, this sequence maintains the primary and secondary structure common to all eukaryotic 16S-like rRNA structures, while truncating sequences found within the eukaryotic variable regions. Comparison of the *T. foetus* 16S-like rRNA sequence with that of human, yeast, *Plasmodium berghei*, and *Trichomonas vaginalis* defined variable regions where the *T. foetus* sequence differed substantially from the other sequences examined. Using ^{32}P-labeled oligonucleotide probes prepared from these regions, we have been able to detect as few as 10 parasites in slot blot analyses without *in vitro* target amplification and without cross hybridization to the nucleic acids of the other trichomonads or the bovine host. *In vitro* amplification allows detection of rRNA from less than the equivalent of a single parasite. Methods are being developed to extract nucleic acids from preputial samples in a form suitable for reliable, routine amplification *in vitro*.

C 115 SUGGESTIVE EVIDENCE FOR A ROLE OF IFN-γ AND A TRYPANOSOMAL COMPONENT IN THE INDUCTION OF *T.BRUCEI* ASSOCIATED IMMUNOSUPPRESSION, A.Darji, M.Sileghem, L.Brys and P.De Baetselier. Laboratory of Cellular Immunology, V.U.B., Paardenstraat 65, 1640 St Genesius Rode, Belgium.

Profound immunosuppression of both T and B cell response has been observed during the experimental *T.brucei* infection. Infected macrophages play a key role in the observed T cell unresponsiveness through two different suppressive mechanisms namely, a prostaglandin (PG)-dependent suppressive mechanism which blocks the IL2 secretion and a PG-independent mechanism which interferes at the level of IL2 receptor expression (1). By using an *in vitro* simulation model, we have mimicked the generation of suppressive macrophages involved in *T.brucei*-induced suppression of T cell unresponsiveness. A selective PG-independent suppression of IL2 receptor expression on lymph node cells (LNC), could be obtained by the macrophage cell line 2C11-12 pulsed with *T.brucei* lysate (2). These pulsed macrophages also trigger lymphocytes to a faster and elevated secretion of macrophage activating factors such as interferon-gamma (IFN-γ). The elevated secretion of IFN-γ was found to be responsible for the hyporesponsiveness of T cells. Indeed, addition of anti-IFN-γ antibodies to cocultures with suppressive macrophages restored the Con-A induced T cell proliferation and reexpression of IL2 receptors. Inoculation of anti-IFN-γ antibodies to *T.brucei* infected animals abolished the *T.brucei* associated immunosuppression. . This *in vitro* model was further adopted to identify the trypanosomal component(s) that render resting macrophages suppressive. By using gel filtration techniques (FPLC), a single protein of 70 kDa was identified that induces resting macrophages towards a suppressive state both *in vivo* and *in vitro*.

Collectively, the following mechanism can be proposed for *T.brucei*-induced suppression of T cell responsiveness. (i) Following interaction with a 70 kDa *T.brucei* component macrophages secrete PG and stimulate T cell to secrete high levels of IFN-γ. (ii) PG inhibits IL2 secretion while IFN-γ plays a role in the down-regulation of the IL2 receptor.

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C 116 SITE-DIRECTED MUTAGENESIS OF THE HYPOXANTHINE-XANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE GENE OF PLASMODIUM FALCIPARUM, Richard L. Davis, Jr., Geetha Vasanthakumar, Department of Biochemistry, Southern Research Institute, Birmingham, AL 35255

Previous experiments conducted in our laboratory resulted in the cloning and expression of a hypoxanthine-xanthine-guanine phosphoribosyltransferase (HXGPRT) encoding cDNA from the malarial parasite Plasmodium falciparum. Using a modification of the Kunkel mutagenesis procedure we have introduced several base-pair alterations within a conserved portion of the HXGPRT thought to represent the purine substrate binding site of the protein. To date we have identified two residues within the putative purine binding site (Arg-80-->Tyr, Gly-81-->Lys) that, when altered to their corresponding human HGPRT equivalents, result in complete abolition of parasite HXGPRT activity. In addition we have mimicked the effects of HGPRT deficiency in humans (Lesch-Nyhan syndrome) by converting the parasite Asp-204 residue to its' human equivalent Tyr and converting Asp-211 to Gly. These alterations also result in complete loss of P. falciparum HXGPRT activity. Further mutagenic alterations of the malarial HXGPRT coding region will provide useful information regarding the purine substrate specificity of the enzyme and should assist in the design of rational inhibitors specific for the parasite protein. This work is supported by the UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases.

C 117 AMPLIFICATION OF THE M2 GENE OF RIBONUCLEOSIDE DIPHOSPHATE REDUCTASE IN HYDROXYUREA RESISTANT LEISHMANIA, Siegfried Detke, Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND 58201.

Leishmania mexicana amazonensis was obtained which was thirteen-fold more resistant to the ribonucleoside diphosphate reductase inhibitor hydroxyurea by gradual selection against increasing concentration of this compound. These cells possessed an extrachromosomal circular DNA of 45 kilobases which was not detected in either wild type or cells which had lost resistance to hydroxyurea. The M2 gene of ribonucleoside diphosphate reductase was mapped to this extrachromosomal DNA by hybridization with a mouse M2 cDNA probe. Both the promastigote and intracellular amastigote stage appeared to possess and be dependent on the enzyme ecoded in this gene for growth.

C 118 AN RFLP MAP OF THE PLASMODIUM FALCIPARUM GENOME: RECOMBINATION FREQUENCIES AND EVIDENCE FOR FAVORED LINKAGE GROUPS IN GENETIC CROSS, Stephen A. Dolan, Annie Walker-Jonah, Robert W. Gwadz, Lindsey J. Panton and Thomas E. Wellems, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institues of Health, Bethesda, MD 20892.

We report a genetic linkage map of the P. falciparum genome, using inheritance patterns of nearly ninety restriction fragment length polymorphism (RFLP) markers in the progeny of a genetic cross. RFLP markers were assigned on each of the fourteen nuclear chromosomes. Genetic recombination between parental clones was detected with RFLP markers in each of the sixteen progeny clones. Inheritance patterns in the progeny clones suggest that linkage groups on chromosomes 2, 3, 12 and 13 were favored in this genetic cross. Analysis of chromosome crossover frequencies from five of the chromosomes yields an approximate chromosome-map unit as 15-30 kb (15-30 kb/centimorgan).

C 119 HETEROLOGOUS EXPRESSION AND KINETIC CHARACTERIZATION OF THE MAJOR CYSTEINE PROTEASE FROM *TRYPANOSOMA CRUZI*,

Ann E. Eakin[1,2], Guenter Harth[3], James H. McKerrow[2], and Charles S. Craik[1], Departments of Pharmaceutical Chemistry[1] and Pathology[2], University of California, San Francisco, CA 94143-0446; Department of Immunology and Infectious Disease[3], Palo Alto Medical Foundation, Palo Alto, CA 94301

A general method for cloning thiol proteases was developed using the polymerase chain reaction (PCR). A gene fragment was amplified using this method and used as a probe to isolate a full-length copy of the gene. The gene was characterized and then heterologously expressed in *Escherichia coli*. The gene was initially expressed as an inactive, insoluble fusion protein to approximately 5% of the total cell protein. The fusion protein was readily purified, solubilized in urea and successfully refolded to produce a poly-protein which processed autocatalytically to yield approximately 1 - 2 mg of active protease per OD per liter of bacterial culture. The processed form of the recombinant protease had an N-terminal sequence identical to that of the mature form of the native protease purified from *T. cruzi*. This suggests that the recombinant protease possesses the requisite specificity and activity to correctly process the proform of the protease *in vitro*. Kinetic assays with peptide substrates demonstrate that the substrate specificity and kinetic parameters for the recombinant protease are consistent with those of the native protease. The proteolytic activity of the recombinant protease is enhanced by dithiothreitol, inhibited by leupeptin, TLCK, and E64 but is unaffected by PMSF, pepstatin, and 1,10-phenanthroline. More specifically, the enzyme was inhibited by the fluoromethyl ketone inhibitor, Z-Phe-Arg-FMK, which inhibits replication and differentiation of *T. cruzi* within mammalian cells in culture.

C 120 SUPRESSION OF *THEILERIA ORIENTALIS* INFECTION IN CALVES BY CO-INFECTION WITH THE HEMOPROTOZOAN PARASITES *BABESIA BOVIS* OR *BABESIA BIGEMINA* OR THE RICKETTSIA *ANAPLASMA MARGINALE*, Kevin R. Gale, Graham L. Leatch, Fernando Parrodi, Brian V. Goodger and Ian G. Wright, CSIRO Division of Tropical Animal Production, Private Mail Bag 3, Indooroopilly, Queensland 4068, Australia.

Intact calves infected with the hemoprotozoan parasite *Theileria orientalis* and uninfected control calves were infected with the rickettsia *Anaplasma marginale*. The *T. orientalis* infected animals reacted less severely to *A. marginale* infection and peak parasitaemia was delayed relative to the *T. orientalis* free animals. Also, upon infection with *A. marginale*, the *T. orientalis* parasitaemias decreased sharply and remained low until after the *A. marginale* parasitaemias had peaked at which time the *T. orientalis* parasitaemias increased rapidly to the pre-*A. marginale* infection level. Following the complete resolution of *A. marginale* infection, the *T. orientalis* infected animals were challenged with virulent organisms of the hemoprotozoan parasite *Babesia bovis*. *T orientalis* parasitaemia again decreased to a very low level as the *B. bovis* parasitaemia increased and then returned to the pre- *B. bovis* infection level after resolution of the peak of the *B. bovis* infection. The *B. bovis* recovered calves were subsequently challenged with *Babesia bigemina* virulent organisms. Again the *T. orientalis* parasitaemias were supressed for the duration of patent *Babesia* infection. This observed antagonistic interaction of widely different hemoparasites is of significance in naturally occuring co-infections and may also serve as a good experimental model for the study of parasite-parasite-host interactions and the mechanisms of immunity of the host to these parasites.

C 121 MOLECULAR CHARACTERIZATION OF A *TRYPANOSOMA BRUCEI* PROTEIN KINASE GENE FAMILY. Michael Gale Jr. and Marilyn Parsons, Department of Pathobiology, University of Washington, Seattle, WA and Seattle Biomedical Reasearch Institute, 4 Nickerson St. Seattle, WA 98109

Protein kinases play a pivotal role in higher eukaryotic cellular signal transduction by mediating such events as communication with the extracellular environment and regulation of cellular differentiation and proliferation. The current study was undertaken to clone and characterize *T. brucei* protein kinase genes and gene products. Using PCR technology employing degenerate oligonucleotide primers complementary to conserved regions within the catalytic domain of protein kinases we were able to clone from *T. brucei* genomic DNA several 33 bp clones with deduced amino acid (AA) sequences characteristic of protein kinases. An oligomer complementary to one of these was used in conjunction with an oligo encoding the 5' mRNA spliced leader sequence to amplify and clone the 5' end of the homologous cDNA. This clone detects two genes by Southern analysis, which were in turn isolated from a genomic library. Restriction analysis and DNA sequencing show the two genes to be very similar, yet distinct. The sequence of the clone corresponding to the original cDNA was obtained and found to contain an open reading frame of 1.3 kb encoding a 48 KDa protein which includes all 11 protein kinase conservation regions (Hanks et al., Science, 241, 1988). It shows the highest overall AA sequence homology with NIM-A G2 specific protein kinase, ribosomal S6 kinase 2 alpha and protein kinase C. The catalytic domain runs from AA 18-278 and is followed by 153 AAs that show some homology to human pleckstrin. Transcripts from this protein kinase gene are present in both blood and procyclic form *T. brucei*. This gene will be expressed in *E.coli* for characterization of substrate specificity. Comparative AA sequence homologies will be discussed.

C 122 ALTERED PERMEABILITY IN HOST CELL MEMBRANES INDUCED BY P. FALCIPARUM. Annette M. Gero and Joanne M. Upston. School of Biochemistry and Molecular Genetics, University of New South Wales, Kensington, NSW 2033. Australia.

Following invasion with the intraerythrocytic malarial parasite, *Plasmodium falciparum* , new permeability pathways are induced in the host erythrocyte membranes (Sherman 1988). Recently we have partially characterised the parasite-induced component of nucleoside transport. This component is insensitive to specific inhibitors of mammalian nucleoside transport, such as nitrobenzylthioinosine (NBMPR) (Gero et.al., 1989). The induced component of nucleoside transport in the host erythrocyte membrane comprises approximately 60% of the total transport in the infected cell and is stage-specific in that it is detected primarily in the trophozoite. It was found to be non-saturable at high concentrations of adenosine (i.e. millimolar range) and kinetic studies have indicated a large component of non facilitated influx. This was not inhibited by the oxidising agent, diamide at concentrations up to 1mM, nor was it a sodium (energy) dependent process. However, it appeared that there was some requirement for D-glucose as adenosine transport in infected cells did not proceed optimally with glucose absent. The parasite induced transport in *P. falciparum* infected erythrocytes also appeared to be more sensitive than uninfected erythrocytes to inhibition by the sulphydryl reagent, *p*-chloromercuribenzoyl sulfonate (PCMBS) (0.1-1mM) and various other reagents, such as 1mM quinine (inhibitor of the Gardos channel) and 0.5-1mM DIDS and furosemide (anion channel inhibitors).

Further study of the biochemical differences in nucleoside transport between malaria infected and normal erythrocytes will contribute to a more complete understanding of the manner by which membrane permeability is altered in Plasmodium infections.

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C 123 EXPRESSION OF PROTEASES DURING TRYPANOSOMA CRUZI DIFFERENTIATION. S. Goldenberg, M.C. Bonaldo, L.N. d'Escoffier, J.M. Salles, C.M. Lowndes, A.C. Murta & J. Scharfstein. FIOCRUZ, Av. Brasil 4365, Rio de Janeiro, RJ, 21040, Brasil

Study of T.cruzi metacyclogenesis (transformation of epimastigotes into metacyclic trypomastigotes) under chemically defined conditions, showed that the expression of a group of acidic polypeptides (Mr 45-50 kDa) is increased on adhesion of epimastigotes to the culture vessels. This group of acidic polypeptides is homologous to Gp57/51, a T.cruzi major antigen characterized as a cysteine proteinase. This proteinase is made up of several isoforms and is developmentally regulated: its expression is 2-to-5 times higher in epimastigotes than in trypomastigotes. Investigation of proteinase activities during T.cruzi metacyclogenesis showed that, in addition to the aforementioned cysteine-proteinase, two metallo-proteinases were detected: A 65kDa enzyme is specifically expressed by metacyclic trypomastigotes, whereas the 52kDa proteinase is constitutively expressed. The observation that proteases are developmentally regulated during metacyclogenesis, suggests that these enzymes might be important for T.cruzi differentiation. Accordingly, this process is inhibited by proteinase inhibitors.

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C 124 THE DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE GENE OF *CRYPTOSPORIDIUM PARVUM*. Lisa Goozé, Carolyn Petersen, Kami Kim, Jiri Gut and Richard G. Nelson. Parasitology Laboratory, San Francisco General Hospital, Depts. of Medicine and Pharmaceutical Chemistry, UCSF, 94143-0811. *Cryptosporidium parvum* is a zoonotic enteropathogen and the causative agent of severe, life-threatening diarrheal disease in AIDS and other immunocompromised patients. Currently, there is no effective chemotherapy for cryptosporidiosis. Curiously, the common DHFR inhibitors trimethoprim and pyrimethamine, which are efficacious treatments for infections caused by closely related coccidian parasites, are not effective against *C. parvum*. As a first step in an effort to understand the basis of cryptosporidial resistance to these folate analogs, and to develop a recombinant enzyme-based assay to identify effective DHFR inhibitors, we have isolated and sequenced genomic clones encoding *C. parvum* DHFR-TS. Current nucleic acid sequence reveals a single, continuous 1.5 kb open reading frame whose deduced amino acid sequence predicts a bifunctional enzyme containing a 170 amino acid N-terminal DHFR domain connected by a 61 amino acid junction peptide to a 270 amino acid C-terminal TS domain. The sequence of the extreme 3' end of the TS coding region (corresponding to ca. 20 amino acids) remains to be determined. Comparison of the deduced *C. parvum* DHFR- and TS-domain sequences with sequences present in the SwissProt database indicate that the DHFR domain is most similar (33% identity) to mammalian monofunctional DHFRs while the TS domain sequence is ca. equally similar (56% identity) to mammalian TS enzymes and to the TS domains of the *P. falciparum* and *P. chabaudi* bifunctional enzymes. Genomic Southern and electrophoretic karyotype analyses using the cloned DHFR-TS gene as an hybridization probe indicate that the *C. parvum* enzyme is encoded by a single copy gene which maps to a 1,200 kb chromosome.

C 125 TRANSFER RNA IMPORT INTO TRYPANOSOME MITOCHONDRIA: IDENTIFICATION AND CHARACTERIZATION OF A PUTATIVE IMPORT INTERMEDIATE, Kathy Hancock, Allen J. LeBlanc, David Donze, and Stephen L. Hajduk, Department of Biochemistry, University of Alabama at Birmingham, Birmingham, AL 35294
The mitochondrial tRNAs of *Trypanosoma brucei* are encoded by nuclear genes and therefore must be imported into the mitochondrion. Typically, RNAs that function in the mitochondria are encoded by the mitochondrial DNA. But it is becoming evident that there are exceptions to this rule. Evidence indicates that several other protozoans; Leishmania, Tetrahymena, and Paramecium; an algae, Chlamydomonas; and several higher plants; Phaseolus, Solanum, and Triticum; import at least some of their mitochondrial tRNAs. In addition, in mammalian cells the RNA components of the mitochondrial RNA processing enzymes, RNase P and RNase MRP, are nuclear encoded and imported into the mitochondria. In spite of the accumulating evidence that RNA import into mitochondria is phylogenetically widespread and that the import of RNA appears to be essential for cellular processes, virtually nothing is known about the mechanism of RNA import. We have identified and characterized mitochondrial precursor tRNAs that are intermediates along the import pathway. These precursor tRNAs hybridize to cloned nuclear tRNA genes, label with α-^{32}P CTP using yeast mitochondrial nucleotidyltransferase, and are processed to mature tRNAs by yeast mitochondrial RNase P. *T. brucei* mitochondrial extracts contain a RNase P activity capable of processing a prokaryotic tRNA precursor as well as the *T. brucei* tRNA precursors. We are currently developing an *in vitro* RNA import system using isolated mitochondrial vesicles and the precursor tRNAs as the substrate for import.

C 126 **CHARACTERISATION OF PROTEIN KINASES FROM TRYPANOSOMA BRUCEI WHICH AUTOPHOSPHORYLATE IN VITRO.** **Geoff Hide, Karen Keith, Andy Tait.** Wellcome Unit of Molecular Parasitology, Glasgow University, Scotland, U.K. We have developed a technique for investigating protein kinases which are capable of autophosphorylation. Autophosphorylation has been implicated as an important control mechanism in signal transduction and growth regulatory pathways in mammalian cells. Autophosphorylation is assayed by the fractionation of trypanosome lysates on an IEF gel followed by in situ incubation with a ^{32}P labelled NTP as the phosphate donor. Kinases appear as radio-labelled bands. We have identified a series of autophosphorylating protein kinases in both bloodstream and procyclic stage T.brucei which require either ATP or GTP as the phosphate donor. Using ^{32}P ATP as a phosphate donor, seven bands were identified in bloodstream T.brucei and 1 band in the procyclic stage. Excision of these bands followed by SDS-PAGE indicated that the molecular size of each band was 60kd. Addition of mammalian Epidermal Growth Factor (EGF) to the samples from bloodstream trypanosomes produced a further band which was also 60kd. Evidence obtained by western blotting using antiphosphotyrosine antibodies indicates that these polypeptides are all phosphorylated on tyrosine. Using ^{32}P GTP as a phosphate donor in the presence of excess unlabelled ATP, four autophosphorylated polypeptides were identified in both bloodstream and procyclic trypanosomes. Analysis of their molecular size indicated that all were 90kd in size. Thus, we have identified at least 2 classes of kinases which are capable of autophosphorylation in vitro. At least in the case of the 60Kd kinases, their properties of stage difference and EGF stimulation, suggests that they may play a role in growth regulatory signal transduction in T.brucei.

C 127 ASSOCIATION OF DIROFILARIA IMMITIS CYSTEINE PROTEINASE ACTIVITY WITH THE MOLT OF INFECTIVE-STAGE LARVAE, W. Garrett Hunt, Jennifer K. Richer, Judy A. Sakanari, and Robert B. Grieve, Department of Pathology, Colorado State University, Fort Collins, CO 80523
Third-stage larvae (L3) of the nematode Dirofilaria immitis were cultured in vitro with fluoromethyl ketone derivatives to determine the effect of cysteine proteinase inhibitors on the L3 to fourth-stage (L4) molt. None of the L3 cultured in 0.1, 0.2, 0.6, and 1.0 mM benzyloxycarbonyl (z)-phe-alaCH$_2$F molted to L4 by 128 hr. In each successively lower concentration of inhibitor, larvae were more motile and more L3 had initiated, but not completed, the molt. In total, four fluoromethyl ketone inhibitors, z-phe-argCH$_2$F, morpholine urea (mu)-homo-leu-pheCH$_2$F, mu-tyr-o-methyl-homo-pheCH$_2$F, and mu-phe-homo-pheCH$_2$F, were tested for their effects on the L3 to L4 molt. Seventy-seven percent of the L3 in 0.2 mM z-phe-argCH$_2$F had molted by 144 hr in vitro, while no more than 1% had molted in the three other inhibitors. L3 remained alive but did not complete the molt. Cysteine proteinase activity in L3 soluble extract was evaluated with a synthetic peptide substrate and fluoromethyl ketone derivatives. The pattern of inhibition observed for the hydrolysis of synthetic peptide substrate by L3 soluble extract was the same as for the molt of L3 to L4. Electron microscopy revealed that L3 cultured in z-phe-alaCH$_2$F had a clearly visible L4 trilaminate epicuticle beneath the L3 epicuticle, implicating cysteine proteinase activity in the exsheathment of the L3 cuticle rather than synthesis of the L4 epicuticle.

C 128 TRICHINELLA SPIRALIS: ANALYSIS OF CELLULAR PROCESSES LEADING TO THE INFECTED CELL PHENOTYPE, Jasmer D.P. Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164

Trichinella spiralis is an intracellular nematode parasite of mammalian skeletal muscle cells. Infection by the parasite induces the reduction of muscle specific proteins to less than 1% of normal muscle cells. Experiments demonstrated that the relative transcript abundances for myofibrillar proteins were reduced to less than 1% in infected cells compared to normal muscle. In addition, transcript levels of muscle specific transcription factors, Myo D1 and myogenin, were reduced to about 1 and 10%, respectively in the infected cell, while in preliminary studies transcripts for the transcription antagonist ID were increased by 2-5 times. These results suggest how the parasite could indirectly induce dedifferentiation of the infected cell via altered expression of transcriptional regulatory factors. In other experiments it was shown that nuclei of infected cells incorporate ^{3}H-thymidine over a restricted period of time consistent with the infection inducing a regeneration response. However, X-irradiation of muscle with 2 krads (sufficient to block muscle regeneration) failed to block labeling of nuclei or normal development of the infected cell. If confirmed, these results could have important implications regarding host cellular processes leading to the infected cell phenotype.

C 129 **FORMATION AND EXPRESSION OF COMPLEX MOSAIC GENES AMPLIFIES THE SURFACE ANTIGEN REPERTOIRE OF *TRYPANOSOMA BRUCEI*. S.M. Kamper and A.F. Barbet, University of Florida, Gainesville, FL 32611.**

Trypanosoma brucei evades the immune response of its mammalian host by antigenic variation in the major surface glycoprotein (VSG). Each trypanosome has a repertoire of about 1000 VSG genes many of which exist in families of partially homologous genes. Family members may undergo partial gene conversion to produce transiently expressed genes which are a mosaic of two or more basic gene copies. Mosaic genes previously reported for *T. brucei* have been simple constructs in contrast to the complex mosaic genes with pseudogene involvement seen in *T. equiperdum*. Correlation between the formation of complex mosaic genes and surface epitope variation has not been clearly demonstrated, although point mutations occurring in *T. equiperdum in vitro* resulted in loss of specific epitopes. We examined the generation of diversity in four, *in vivo* derived, antigenically related clones of *T. brucei* by sequencing VSG cDNA from each of the four clones and all five related genomic copies in the WaTat 1.1 progenitor organism. Each expressed VSG gene was a different mosaic of basic copy genes; three were complex mosaics of multiple fragments of at least three basic copy genes. At least two basic copy genes involved in mosaic formation were pseudogenes. Point mutations were a minor component in VSG variability; three nucleotide mutations were observed and only one of these resulted in an amino acid change. Loss of specific surface epitopes correlated with sequence differences generated by partial conversion and mutation. We conclude that, *in vivo*, expression of mosaic VSG genes results in antigenic diversity beyond that encoded in the basic repertoire and may be a primary function of multigene VSG families and the main mechanism for survival late in infection after the host has been exposed to the basic VSG repertoire.

C 130 THE PROTECTIVE P.yoelii 235kD RHOPTRY PROTEIN IS ENCODED BY A MULTIGENE FAMILY. Jane Keen, Katharine Sinha, Carolyn Owen, Solabomi Ogun, Neil Brown and Anthony Holder. Parasitology Division, National Institute of Medical Research, The Ridgeway, London NW7 1AA, England. Mice immunised with the P.yoelii 235kD rhoptry protein or with passively transferred monoclonal antibodies are protected against virulent challenge. Immunisation alters the invasion characteristics of the virulent YM strain by restricting the parasite to reticulocytes followed by clearance after 15 days. Sequence data from two P.yoelii genomic clones, Southern blots and chromosome blots have shown that the 235kD rhoptry protein is encoded by a multigene family. A comparison of the DNA and derived protein sequences of two large genomic fragments has revealed regions of extreme homology but has also shown that the two copies contain unique sequences. Southern blots and chromosome blots suggest that the gene family contains at least four members which map to different chromosomes.

C 131 EXPRESSION OF FOREIGN GENES IN TRYPANOSOMA CRUZI AND LEISHMANIA MEXICANA, John M. Kelly, Helena M. Ward and Giles Kendall, London School of Hygiene and Tropical Medicine, London WC1E 7HT.

The genes that encode the glycosomal enzyme glyceraldehyde 3-phosphate dehydrogenase (gGAPDH) of Trypanosoma cruzi are arranged as a direct tandem repeat. Transient transfection assays based on expression of chloramphenicol acetyl transferase (CAT) have localised the gGAPDH promoter to a short stretch of DNA upstream of the 5'-proximal gene. This region of DNA was used to construct two expression vectors which upon electroporation conferred G418-resistance on T. cruzi cells. Integration of transfected sequences into the T. cruzi genome is stable and occurs predominantly by a mechanism involving insertional duplication. Co-transfection procedures allow the expression of additional genes in transformed cells. The vectors also proved capable of stably transforming Leishmania mexicana implying a functionally conserved mechanism for the expression of the gGAPDH genes. These expression vectors should have numerous uses for investigation of genetic, biochemical and immunological aspects of these medically important organisms.

This work was funded by the Wellcome Trust and the Medical Research Council. G.K. is in receipt of a fellowship from the Royal Society.

C 132 RNA POLYMERASE ACTIVITY IS ASSOCIATED WITH PURIFIED VIRUS-LIKE PARTICLES OF *TRICHOMONAS VAGINALIS*, M.A. Khoshnan and J.F. Alderete, Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-7758

Only isolates of *Trichomonas vaginalis* with the ability to undergo phenotypic variation are persistently infected with an RNA virus-like particle having genome size of approximately 5 kilobases. The presence of these RNA genomes correlates with surface expression of a major immunogen known as p270. Virus-like particles were purified on a sucrose gradient and assayed in vitro for polymerase activity. Incorporation of radiolabelled CMP an UMP were detected into TCA precipitable viral transcripts. RNA of genomic length along with subgenomic transcripts were synthesized in vitro. The nascent transcripts hybridized to RNA extracted from the purified viral particles in northern blot analyses. Similar transcripts were also observed in total RNA from infected cells. These results suggest that transcriptase activity is associated with assembled virus-like particles found in some isolates of *T. vaginalis*.

C 133 THE HSP70 GENES OF LEISHMANIA AMAZONENSIS. Pamela J. Langer, Jeffrey H. Bock, William C. Hokanson, Dorly Piske, Denise R. Prugh and Teresa Ambrose, Department of Molecular Biology, University of Wyoming, Laramie, WY 82071. In order to study the heat shock response and transcriptional regulation in Leishmania, we have chosen to analyze the hsp70 gene locus of Leishmania amazonensis. Genomic and cDNA libraries from late log phase promastigotes have been constructed and several phage, cosmid and cDNA clones have been isolated. Sequence analysis of genomic and cDNA clones has shown that, in comparison with hsp70 genes from L. major and L. donovani, the coding sequence is highly conserved; however the intergenic regions and the 5′ and 3′ untranslated regions differ substantially. Analysis of the cDNA clones has revealed two poly A addition sites in otherwise identical 3′ untranslated regions, yielding 3′ untranslated regions of either 930 nt or 1031 nt long. For one cDNA clone, the presence of part of the spliced leader sequence indicates that the 5′ untranslated region for this clone is 144 nt long. Several cosmid clones covering an area of 134 kb have been isolated and restriction mapped in order to define the L. amazonensis hsp70 gene locus. Seven genes are arranged in a tandem 3.5 kb repeat unit. Although an isolated gene copy is frequently found within a few kb of a gene cluster in trypanosomatids, we have found no evidence for such a gene copy within, at minimum, 29 kb of the seven tandem repeats. However, a single, more divergent eighth gene is found at another locus which must be at least 36 kb away from the main locus.

C 134 DIFFERENTIAL GENE EXPRESSION IN *LEISHMANIA MAJOR*. S. Lindfield, H. Flinn, B. Kelly, and D.F. Smith, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, U.K. SW7 2AZ.

A number of genes have been isolated that show either unique or increased expression in infective (metacyclic) promastigotes of *Leishmania major*. These genes were detected by differential screening of a cDNA library which produced four cDNA clones each recognising multiple transcripts, expressed at developmentally distinct stages of the parasite life cycle (Coulson and Smith,1990 *Mol. Biochem. Parasitol. 40*: 63-75)

LmcDNA 14 recognises a major transcript of 2Kb which is specific to metacyclic promastigotes. **LmcDNA 2** recognises a 6Kb mRNA, which is upregulated in infective metacyclics, and shows a further 50 fold increase in amastigote forms. The gene encoding this RNA is part of a tandemly repeated gene family found on chromosome 22. **LmcDNA 16** recognises 5 differentially regulated transcripts: all five show either increased or unique expression in metacyclic promastigotes, with two being present exclusively in metacyclics and amastigotes. These genes are polycistronically transcribed, and encode a protein(s) found on the surface of infective forms of the parasite.

Recent data will be presented regarding the expression and function of these genes and their products, in *Leishmania major*.

C 135 IS TNF-α RESPONSIBLE FOR LETHALITY ENCOUNTERED IN TRYPANOSOMIASIS-TREATED ANIMALS ? R. Lucas, E. Bajyana Songa, S. Magez, A. Darji, R. Hamers and P. De Baetselier. Laboratory of Cellular Immunology, V.U.B., Paardenstraat 65, 1640 St-Genesius-Rode, Belgium.

Sera obtained from Trypanosoma evansi infected rabbits or mice that underwent a 24h treatment for trypanosomiasis with different trypanocidal agents proved to be toxic when transferred to naïve mice. Since the sera of the infected/treated animals showed a massive increase in Tumor Necrosis Factor alpha (TNF-α) levels, the possible role of this cytokine in the observed lethality was investigated. Injection of a neutralizing anti-TNF-α monoclonal antibody in the acceptor mice, totally inhibited the observed lethality, suggesting an important role for TNF-α in this process. Since trypanolysis results in the release of trypanosome fragments in the sera of the infected animals, we subsequently investigated wether soluble extracts of different extracellular and intracellular trypanosomes can induce TNF-α production in vitro or in vivo. The lysates of all the extracellular trypanosomes, but not that of the intracellular T. cruzi, potently induce TNF-α both in vitro and in vivo. In the case of T.bruceï bruceï a 40 kdal glycoproteïn is mainly responsible for the observed TNF-α induction. Collectively, these results indicate a potential role of TNF-α in the observed lethality.

C 136 GENETIC ANALYSIS OF THE *LEISHMANIA DONOVANI* SECRETORY ACID PHOSPHATASE. Cathleen McCarthy-Burke[1], David J. Mallinson[1], Victoria H. Mann[1], Robert Olafson[2], Dennis M. Dwyer[1].[1] Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD 20892.
[2] Department of Biochemistry, University of Victoria, Victoria, British Columbia, V8W 2Y2, Canada.
The biochemistry of the *L. donovani* secretory acid phosphatase (SAcP) has been characterized extensively. To discern its developmental expression, our current investigations focus on identification of the gene(s) encoding this enzyme. The SAcP was purified to homogeneity and subjected to amino acid sequence analysis. Codon-biased oligonucleotides were synthesized based on the N-terminal and an internal amino acid sequence of the SAcP. In a polymerase chain reaction, these primers produced a unique 400 base pair fragment (400-mer) with an open reading frame bearing 100% homology to the amino acid sequence of the purified SAcP. The 400-mer was used as a probe for both Southern analyses and screening a λgt11 cDNA library. This probe hybridized to three distinct bands of 3, 4.2, and 5.5kb in PstI digested genomic DNA. These have been cloned into the plasmid vector pSport1 for sequencing. Tertiary screening of a cDNA library with this probe resulted in 43 positive clones. These clones were confirmed by immunoscreening with a rabbit antibody against purified *L. donovani* SAcP. Following restriction analysis, the insert DNA will be subcloned into pUC18 and sequenced. Characterization of this gene will facilitate studies of its expression and regulation during parasite development.

C 137 cdc2-LIKE GENES IN TRYPANOSOMES AND LEISHMANIA. Jeremy C. Mottram, Andrew Tait, Brian R. Shiels, Jane Kinnaird and J. David Barry.
Wellcome Unit of Molecular Parasitology, University of Glasgow, Glasgow, UK.
During its life-cycle the trypanosome has rapidly dividing forms which establish infection and non-dividing forms preadapted to survive in the new environment of the next host. There appears to be an integral link between the life-cycle and the cell cycle in trypanosomes as the non-dividing forms are arrested in the G1 phase of the cell cycle, with the release of the cell cycle block being concomitant with infection of the new host and differentiation to the next stage of the parasite. p34cdc2 protein kinase is a central component in the control of the eukaryotic cell cycle and is highly conserved in evolution. We have isolated two single copy cdc2-like genes from Trypanosoma brucei, coding for 34 and 39 kDa proteins and a p34cdc2-like gene from Leishmania mexicana. Antisera raised against either fusion proteins of the expressed genes, or peptides predicted from gene sequence, are being used to analyse the distribution of the proteins in different life-cycle stages. These sera cross react with a number of polypeptides and it is clear that in trypanosomes there is a family of related cdc2-like proteins, that may possibly be involved in cell cycle control. An analysis of these cdc2-like proteins and their associated polypeptides (eg cyclins) should provide insights into the way the cell cycle is controlled in trypanosomes.

C 138 PCR-FACILITATED CLONING OF THE TRIOSE PHOSPHATE ISOMERASE GENE OF THE PRIMITIVE EUKARYOTE *Giardia lamblia*. Michael R. Mowatt, Timothy C. Howard and Theodore E. Nash, Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892
Giardia lamblia, one of the most common pathogenic protozoan parasites of man, colonizes the upper small intestine causing diarrhea, weight loss and other gastrointestinal maladies. Biochemical studies have shown that the binucleate flagellated trophozoite, which lacks mitochondria, is a microaerotolerant anaerobe capable of generating ATP through glycolysis, but displays neither cytochrome mediated oxidative phosphorylation nor a functional citric acid cycle. As a starting point in evaluating the molecular genetics of metabolically significant enzymes of *G. lamblia*, we examined the gene encoding the well characterized and highly conserved glycolytic enzyme triose phosphate isomerase (TIM). TIM catalyzes the isomerization of D-glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate. In terms of efficiency of energy production TIM is a pivotal enzyme in glycolysis, since only GAP continues along the pathway. Oligonucleotide guessmers designed from conserved regions of TIM were used in the polymerase chain reaction (PCR) to amplify a 246 basepair product from *G. lamblia* genomic DNA. The DNA sequence of the product predicted a polypeptide with homologies to known TIM sequences ranging from 35 to 54 percent. In Northern hybridization the radiolabelled PCR product detected a transcript estimated to be 1000 nucleotides, in good agreement with the RNA size expected for a TIM monomer of about 26 kilodaltons. Variable hybridization of the probe in genomic Southern analysis suggested significant nucleotide sequence divergence among the single copy TIM genes of different *G. lamblia* isolates. The sequences of TIM genes cloned from two divergent isolates were determined and their deduced amino acid sequences compared to TIM from 14 sources that included both prokaryotes and eukaryotes. Sequence analysis of the small subunit ribosomal RNA has resulted in the inference of a phylogenetic tree in which *G. lamblia* represents the earliest diverging lineage in the eukaryotic line of descent (Sogin et al., 1989, Science, 243, 75-77). The TIM comparisons presented here should be useful in further defining the relationship of *G. lamblia* to other eukaryotes and to prokaryotes.

C 139 MAPPING AND CLONING VSG EXPRESSION SITES AND SURROUNDING SEQUENCES IN MEGABASE-SIZED CHROMOSOMES OF *TRYPANOSOMA BRUCEI*. Peter J. Myler, Michael J. Lodes, Bob L. Smiley, Andrew W. Stadnyk, and Kenneth D. Stuart, Seattle Biomedical Research Institute, 4 Nickerson St., Seattle, WA 98109-1651.

Pulsed field gradient electrophoresis (PFGE) analysis of chromosomal DNA from the ISTAR1 serodeme of *T. brucei* has shown a molecular karyotype of numerous minichromosomes (50-150 kb), one or more intermediate-sized (375 kb) chromosomes (I), several megabase-sized chromosomes (M1-M4), in addition to large chromosomes which were not resolved. Mapping studies on chromosomal DNA isolated by PFGE indicates that the M4 and M3 bands consist of single chromosomes, while the M2 band contains two chromosomes. Similarity in restriction pattern suggests that M4 and M3 are homologous chromosomes, despite their difference in size (1.4 vs 1.5 Mb). We have characterized VSG gene expression sites at the telomeres of these chromosomes; T3 and T5 expression sites on M4, T2 and T7 sites M3, and the T6 site on one of the M2 chromosomes. Comparison of ESAG 3, T-LR, 4, 5, 6 and 7 sequences from genomic clones of the T3 and T5 expression sites and cDNAs obtained by PCR amplification of mRNA confirmed that these sites were expressed in VATs 7 and 5, respectively. While the expression sites were similar in sequence, restricted regions of sequence divergence, especially upstream of the putative promoter enabled us to prepare expression-site specific probes for chromosome mapping studies. The relevance of these findings to the regulation of antigenic variation is discussed.

C 140 ISOLATION OF *CRYPTOSPORIDIUM PARVUM* TUBULIN GENES. Richard G. Nelson, Lisa Goozé, Carolyn Petersen and Jiri Gut. Parasitology Laboratory, San Francisco General Hospital and Depts. of Medicine and Pharmaceutical Chemistry, UCSF, 94143-0811. Invasive zoite stages of *Cryptosporidium parvum* and related Apicomplexan parasites lack obvious motile organelles (ie. cilia and flagella) yet they efficiently move over substrata by intermittent but rapid "gliding" motions. The molecular mechanism of gliding motility is unknown; however, it is accompanied by a polarized capping of surface antigen(s) to the posterior pole of the zoite. The capping reaction is thought to be mediated by a subpellicular actomyosin contractile system while the polarity is hypothesized to be determined by the inherent asymmetry of the microtubule cytoskeleton. In order to develop reagents necessary to investigate these hypotheses, we have initiated a project to clone and express *C. parvum* genes encoding microfilament and microtubule proteins. Here we report the isolation and preliminary sequence analyses of genomic clones encoding the *C. parvum* microtubule proteins α- and β-tubulin. Degenerate oligonucleotide probes whose sequences were derived by reverse translation of strongly conserved regions of α- and β-tubulin amino acid sequence identified one α- and three β-tubulin clones in a preliminary screen of ca. 2×10^5 recombinant phage from a *C. parvum* genomic library. The single α-tubulin clone and the largest β-tubulin clone were partially sequenced and the single open reading frames identified unmistakably encoded α- and β-tubulin proteins respectively. FastA searches of the SwissProt database with these partial sequences shows them to be unique and most similar to the α- and β-tubulins of two related sporozoan parasites, *P. falciparum* and *T. gondii*. Genomic Southern and molecular karyotype analyses using the cloned tubulin gene fragments as hybridization probes suggests that *Cryptosporidium* α- and β-tubulins are encoded by single copy, unlinked genes.

C 141 APPLICATION OF THE POLYMERASE CHAIN REACTION TO THE DETECTION OF PYRIMETHAMINE AND PROGUANIL RESISTANT *PLASMODIUM FALCIPARUM*. David S. Peterson[1], Frederick N. Gyang[2], Thomas E. Wellems[1], [1]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda MD 20892; [2]Department of Biochemistry, University of Ghana, Post Office Box 54, Legon, Ghana.

All naturally antifol resistant isolates of *Plasmodium falciparum* have been found to contain point mutations in the active site of the dihydrofolate reductase of the parasite. Differential resistance to pyrimethamine and cycloguanil, the active metabolite of the antifolate antimalarial proguanil, is linked to specific point mutations. A single point mutation (Ser-108 → Asn-108) in the DHFR active site has been linked to pyrimethamine resistance, but has little affect on the sensitivity of the parasite to cycloguanil . Parasites with paired mutations at the active site of the enzyme (Ser-108 → Thr-108 and Ala-16 → Val-16) have been found to be resistant to cycloguanil, but exhibit only a small decrease in sensitivity to pyrimethamine. Additional point mutations increase the level of pyrimethamine resistance and can provide cross resistance to cycloguanil. Mutation specific primers capable of discriminating between wild type and mutant in the PCR have been developed and tested for each of these loci. These primers along with a simple procedure for isolating parasite DNA allow the rapid analysis of parasites from small volume blood samples. We will present details on the design of mutation specific primers, as well as the analysis of samples collected from the Brazilian Amazon. The incidence of the Ser-108 → Asn-108 point mutation in these samples (90%) correlates well with Fansidar failure rates reported from certain states in Brazil.

C 142 DEVELOPMENTAL REGULATION OF CYTOCHROME C REDUCTASE IN TRYPANOSOMA BRUCEI, Jeffrey W. Priest and Stephen L. Hajduk, Department of Biochemistry, University of Alabama at Birmingham, Birmingham, AL 35294

The protozoan parasite *Trypanosoma brucei* cycles between a bloodstream form with a mitochondrion that lacks spectrally detectable cytochromes and an insect procyclic form with a fully functional mitichondrion. The blood stage trypanosomes possess RNA transcripts for the mitochondrially encoded cytochrome *b* and for a nuclear encoded cytochrome *c* at levels that approach those of the procyclic form. Cytochrome *c* expression is regulated mainly at the level of protein stability rather than at the level of translation. To study the developmental regulation of cytochrome *c* reductase in *T. brucei* the complex was first purified from a related trypanosomatid, *Crithidia fasciculata*. Purified complex retained antimycin A sensitivity and catalyzed the reduction of horse heart ferricytochrome *c* in the presence of reduced Coenzyme Q_{10}. The complex contained 2.9 nmoles of heme *b* and 1.4 nmoles of heme c_1 mg^{-1} of protein for a heme *b*:heme c_1 molar ratio of approximately 2:1. Nine major subunits were resolved by SDS PAGE ranging in size from 55 kDa to less than 14 kDa. Subunit V (31.6 kDa) was identified as cytochrome c_1 by the presence of a covalently attached heme. Monoclonal antibodies against subunits II and IV cross-reacted with similar sized proteins in extracts of *T. brucei* procyclic cells but failed to react with any proteins from extracts of *T. brucei* bloodstream cells. When bloodstream *T. brucei* cells differentiated to procyclic forms in vitro, subunits II and IV were first detected by Western blot analysis at about six hours after transfer to 26 °C in procyclic culture medium. The amounts of these two subunits increased over 96 h at a rate approximately equal to that of spectrally detectable cytochrome levels. Because the steady state levels of cytochrome *c* reductase subunits were below the level of detection in bloodstream trypanosomes, we propose that expression of functional cytochrome *c* reductase is not strictly regulated at the level of complex assembly but rather involves the coordinate regulation of the expression of the individual subunit proteins.

C 143 CHARACTERIZATION OF *Babesia bigemina* rRNA GENES AND UTILIZATION OF OLIGONUCLEOTIDE PROBES COMPLIMENTARY TO THE SMALL SUBUNIT rRNA FOR ITS DETECTION IN BOVINE BLOOD, **G. Roman Reddy and John B. Dame, Department of Infectious Diseases, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611.**

Babesia bigemina and *Babesia bovis* are tick-borne, protozoan hemoparasites responsible for bovine babesiosis in tropical areas of the world. Detection of this parasite is routinely done by light microscopy which lacks sensitivity. After acute or primary infections, recovered animals may sustain a subclinical infection which is microscopically undetectable. These animals, known as carrier animals, serve as reservoirs for disease transmission. In an attempt to develop a test sufficiently sensitive to detect parasites in carrier animals as well as patent infections, we have cloned the ribosomal RNA gene units from this parasite. Three distinct ribosomal RNA transcription units designated A, B and C were identified. The complete nucleotide sequence of the small subunit rRNA (SSrRNA) coding region were determined. Units A and B had identical sequences but unit C differs from units A and B at two adjacent positions. The RNA of both sequence types is transcribed in parasites from erythrocyte culture, at a ratio of A/B to C of approximately 4:1. Three synthetic oligonucleotide probes complementary to unique regions of the SSrRNA were identified for its sensitive detection. In about 20 hours, these probes specifically detected as little as 20 pg of total RNA isolated from cultured parasites or total RNA isolated from 100 infected erythrocytes mixed in 20 μl of bovine blood. The parasitemia under these conditions is equivalent to 5×10^{-5} %. These probes did not bind to total RNA or genomic DNA isolated from *B. bovis*, or to bovine leukocyte RNA. The sensitivity was increased to detection of a single parasite when the target SSrRNA was amplified *in vitro*. This work was supported by USAID Grant DAN-4178-A-00-7056-00

C 144 A TOXOPLASMA GONDII RHOPTRY PROTEIN (ROP1) WITH HOST CELL PENETRATION ENHANCING ACTIVITY INCREASES ADHERENCE OF TACHYZOITES TO FIBROBLASTS. Joseph D. Schwartzman and Linda D. Saffer, Department of Pathology, University of Virginia School of Medicine, Charlottesville, VA 22908

ROP1 is a rhoptry protein of the protozoan parasite *Toxoplasma gondii* that is recognized by the monoclonal antibody TG49. This Mab inhibits the phenomenon of host cell penetration enhancement, and ROP1 has therefor been implicated in host cell invasion. The major component of ROP1 has an apparent mobility of 60 kDa on SDS-PAGE as recognized by immunoblot, but a 75 kDa precursor and numerous smaller proteolytic fragments are also detected. We purified ROP1 by means of several chromatographic techniques and assayed the resultant fractions in a bioassay for penetration enhancement, which relies on the specific incorporation of tritiated uracil into parasites which have successfully invaded host cells. Purification of ROP1 by affinity chromatography with Mab TG49 produced relatively pure antigen that induced a small (~2 fold) but reproducible increase of host cell penetration. More impressive quantitative increases in host cell penetration (4-8 fold) were induced by ROP1-containing high molecular weight fractions that were prepared by rapid size exclusion chromatography on a Superose-12™ column (Pharmacia). These fractions contained numerous proteins, and we have shown that similar fractions have phospholipase A activity, which has penetration enhancing activity separate from ROP1. However, Mab TG49 inhibited the penetration enhancement induced by these high molecular weight fractions, implying that ROP1, perhaps in concert with other factors, is involved in the process of host cell penetration. Superose-12™ fractions which demonstrate penetration enhancement increase adherence of *T. gondii* to host cells in parallel with the increase of intracellular parasites, as measured by recovery of radioactivity associated with pre-labeled parasites from extensively washed monolayers of fibroblasts. This adherence is temperature dependent, occurring at 25 °C (a temperature at which *T gondii* does not invade host cells, and adherence can be distinguished from invasion) but not at 0 °C . ROP1 appears to enhance host cell penetration by increasing the adherence of tachyzoites to host cells, which may be of importance in parasite motility and invasion.

C 145 A NUCLEIC ACID BASED TEST FOR DETECTION OF *Fasciola hepatica*. Catherine Shubkin, Michael W. White, Mitchell Abrahamsen, and Stuart Knapp, Veterinary Molecular Biology, Montana State University, Bozeman, MT 59717.

The use of nucleic acid techniques in the diagnosis of parasitic infection has become increasingly widespread. These techniques were used to generate an oligonucleotide probe for detection of *Fasciola hepatica*, the common bile duct fluke, in its snail intermediate host, *Pseudosuccinea columella*. Total RNA isolated from whole liver flukes was used in a polymerase chain reaction (PCR) to isolate and amplify a region of approximately 650 base pairs in the small-subunit ribosomal RNA gene. This portion of the rRNA gene, which contains both highly conserved regions, as well as variable regions, was subcloned and sequenced. In comparison to known small subunit rRNA sequences, a sequence unique to *F. hepatica* was identified.

The *F. hepatica* DNA probe, designated CS4, was shown to specifically hybridize to *F. hepatica* 18s rRNA and not to rRNA of the snail or other unrelated RNAs tested. As few as 5 miracidia may be detected by direct hybridization of the CS4 probe without the need for amplification by the polymerase-chain-reaction. Preliminary studies in infected snails have shown that CS4 is able to detect infected snails by a simple one-step extraction procedure despite the large excess of snail nucleic acid material. Currently, we are undergoing quantitative evaluation of this probe for future use in tracking the course of *F. hepatica* infection in the snail vectors.

C 146 A NEW RETROPOSON-LIKE SEQUENCE IDENTIFIED UPSTREAM OF VSG EXPRESSION SITES IN *T. BRUCEI*. Bob L. Smiley, Michael J. Lodes, Andrew W. Stadnyk, Peter J. Myler and Kenneth D. Stuart, Seattle Biomedical Research Institute, 4 Nickerson Street, Seattle, WA 98109-1651.

The VSG expression site in African trypanosomes consists of a long (>50 kb) coordinately expressed region. The transcripts which characterize the expression site are stage and VAT specific, and are α-amanitin sensitive. A promoter region for this transcriptional unit has been proposed. Upstream of the putative VSG expression site promoter we have identified a new retroposon-like sequence which shares 55% homology to other *T. brucei* retroposon-like sequences (Ingi/TRS). On one megabase-sized chromosome (M4), copies of this sequence have been found upstream of the proposed VSG promoter region on both telomeric ends. Probes which can differentiate between the two copies each hybridize to multiple copies in the genome. Two additional genomic copies have been isolated and sequenced. There do not appear to be sizable open reading frames in any of the four sequenced copies. The significance of these sequences will be discussed.

C 147 CONTRIBUTION OF INTERLEUKIN-4, TNF-α AND IFN-γ TO INDUCTION OF ANTI-LEISHMANIAL MACROPHAGE ACTIVATION. Werner Solbach, Steffen Stenger, Martin Röllinghoff and *Christian Bogdan. Institute for Clinical Microbiology, University Erlangen, Germany and *Cornell Univ. Med. Center, New York, N.Y. 10021.

Destruction of intracellularly living *Leishmania major* amastigotes is achieved by activated macrophages. We have investigated the contribution of IL-4, TNF-α and IFN-γ to the induction of antileishmanial macrophage activation. It was found that as single lymphokine only IFN-γ led to amastigote elimination by peritoneal exudate macrophages. Neither IL-4 nor TNF-α or the combination of both cytokines led to antimicrobial activation. When the macrophages were incubated with concentrations of IFN-γ that by themselves were insufficient for maximum cell activation, it was found that both IL-4 and TNF-α very effectively synergized with IFN-γ for induction of antiparasitic activity. The activation which was achieved when IFN-γ was combined with IL-4 could be blocked not only with antibodies to either of the lymphokines, but also with an antiserum specific for TNF-α, suggesting the involvement of endogeneously generated TNF-α. This was indeed the case, since IFN-γ and IL-4 synergistically induced the endogenous production of TNF-α. Any of the synergistic activities observed presumably lead to the activation of the L-arginine dependent pathway used by the cell for the production of nitrogen oxides as effector molecules for parasite killing since N^G-monomethyl-L-arginine (L-NMMA), a specific inhibitor of this pathway, completely blocked the killing of intracellular parasites.

We conclude that macrophage activation for antiparasitic activity is directed by a complex network of cytokine-interactions, in which IL-4 and TNF-α very effectively synergize positively with low levels of IFN-γ.

C 148 THE LR1 VIRUSES OF *LEISHMANIA* ENCODE AN RNA DEPENDENT RNA POLYMERASE.

K. Stuart, R. Weeks, L. Guilbride, and P. Myler, Seattle Biomedical Research Institute, 4 Nickerson St., Seattle, WA 98109-1651.

We have found RNA viruses (LR1) in *Leishmania guyanensis* and *L. braziliensis* from the Amazon river basin. Most of the viral RNA is double stranded and contained in 32 nm particles. A small fraction of the RNA is single stranded, present in less dense particles. Analysis of cloned LR1 sequences indicated that this RNA corresponds to the (+) strand, suggesting a replication cycle like that of the yeast ScV viruses. Determination of the complete nucleotide sequence has identified two major ORFs that encode the presumptive viral coat protein and the RNA dependent RNA polymerase (RDRP). The RDRP has significant homology to that of the yeast ScV RDRP. In addition, a portion of this ORF is highly conserved among viral strains. The two ORFs overlap and have characteristics suggesting production of a coat/RDRP fusion protein. The 5' end of the viral RNA lacks the SL sequence that is present at the 5' end of all *Leishmania* mRNAs. The 5' 450 nts of the viral RNA contains an ORF that could encode a 72 amino acid protein. However, the lack of the SL sequence and the high conservation of this sequence among viral strains suggests that this region may be involved in initiation of translation, rather than having protein coding function.

C 149 IDENTIFICATION AND TRANSCRIPTIONAL ANALYSIS OF THE GENES FOR HSP60 AND HSP90 IN PLASMODIUM FALCIPARUM, Chiang Syin, Teh-Yung Liu and Neil Goldman, Division of Biochemistry and Biophysics, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892

The malaria parasite has a complex life cycle which includes dramatic changes in its environment during transition between the vertebrate host and the invertebrate vector. Recent findings in Plasmodium falciparum, the most deadly strain of human malaria, indicate that heat shock proteins (hsps), i.e. hsp70, may play a role in the parasite's survival and development. To study the function of other hsps in these parasites, we set out to identify two major proteins, hsp60 and hsp90, in Plasmodium falciparum. Genes for both hsp60 and hsp90 have been isolated from a λgt11 genomic library, and the deduced amino acid sequences show a high degree of homology with hsps from other sources. Northern blot analysis indicates that the transcript for Pfhsp90 is about 3.5 kb and that for Pfhsp60 is about 2.6 kb in size. The level of expression of each of these hsps during different stages of development is being determined.

C 150 IDENTIFICATION, CLONING AND SEQUENCING OF TWO CYSTEINE PROTEINASE GENES FROM **LEISHMANIA PIFANOI**, Yara M. Traub-Cseko, Linda K. Boukai, Ricardo W. Almeida and Diane McMahon-Pratt*, Fundação Oswaldo Cruz, Departamento de Bioquímica e Biologia Molecular, P.O.Box 926 Rio de Janeiro, RJ, 21045, Brazil, *Yale University School of Medicine, Department of Epidemiology and Public Health, 60 College Street, New Haven, CT, 06510.

Leishmania are parasitic protozoa that cause a broad spectrum of disease, that range from cutaneous to visceral leishmaniasis. Cysteine proteinases are believed to be involved in the survival of the parasites within the macrophage, and so provide a useful target for therapeutic attack. We have amplified cysteine proteinase gene fragments from **L.pifanoi** through PCR, utilizing active sites as degenerate primers, and cDNA. Two genes were isolated and sequenced, Lpcysl and Lpcys2, that were shown to be quite different at the level of copy number, genomic organization, chromosome location and level of expression. A genomic library from **L.pifanoi** was screened using the Lpcysl and Lpcys2 500bp fragments cloned into pUC18. In the case of Lpcysl, that has few gene copies, the clones obtained were characterized by the search of diagnostic fragments seen in genomic Southern blots. PstI and KpnI fragments, that should contain the whole gene, were subcloned into pUC, mapped, the location of the genes established, and sequencing is now in progress. Three positive clones were obtained for Lpcys2, that is known to belong to a gene family. The repeat unit was cloned into pUC and M13, for mapping and sequencing. Partial sequencing of the M13 clones confirmed the presence of the cysteine proteinase genes. The pUC clones were mapped and some heterogeneity was observed mostly in intergenic regions. Sequencing is being carried on with the intent of obtaining the complete genomic and intergenic sequences, and of understanding the possible implications of the observed heterogeneities.

C 151 PHYSICAL AND TRANSCRIPTIONAL ANALYSIS OF A CIRCULAR, MULTICOPY DNA IN *LEISHMANIA*.

Cynthia Tripp and Kenneth Stuart, Seattle Biomedical Research Institute, Seattle, WA 98109-1651

Leishmania DNA 1 (LD1) is a 27.5 kb sequence that occurs in one of three genomic arrangements in all *Leishmania* stocks (>100) that we have examined. We have characterized the structural and transcriptional organization of LD1 in *L. infantum* ITMAP 263. In this stock, the LD1 sequence occurs in a tandem array integrated into a 1.5-2.0 megabase chromosome and also in a multicopy, circular DNA which contains two copies of the 27.5 kb sequence arranged as an inverted repeat. The 27.5 kb sequence and the junctions of the inverted repeats have been cloned and mapped. Northern blot analysis indicates that LD1 transcripts are polyadenylated and contain the 35 nucleotide spliced leader sequence at the 5' termini. An initial transcription map of the circular molecule suggests that transcription of LD1 is polycistronic and nine abundant transcripts, which may represent mature messages, are transcribed from adjacent regions of one strand of the 27.5 kb repeat. Several antisense RNAs are encoded on the opposite strand.

C 152 *TRYPANOSOMA CRUZI* STIMULATES HUMAN LYMPHOCYTES TO PROLIFERATE AND PRODUCE CYTOKINES Wesley C. Van Voorhis and Lynn Barrett, Division of Infectious Diseases, Department of Medicine, University of Washington, Seattle, WA 98195. *T. cruzi* has been shown to cause non-specific polyclonal proliferation of lymphocytes after infection *in vivo*. Co-culture of human peripheral blood mononuclear cells (PBMC) with *T. cruzi* CL strain leads to proliferation of lymphoblasts, which peaks on days 5 to 7 after infection. Approximately 15% of lymphocytes in culture undergo blast transformation. The proliferative response of lymphoblasts can be measured by ^3H thymidine uptake, because the parasites appear to incorporate little thymidine, or by FACS analysis of lymphoblasts. By FACS analysis, lymphoblasts from these cultures are (mean % +): 33% B cells (CD19+); 51% T cells (CD3+); 25% CD4+; and 20% CD8+. A mean of 68% of lymphoblasts express MHC class II and IL-2R p55, suggesting both B and T lymphoblasts express these molecules. Anti-MHC class II inhibits the proliferative response of PBMC to *T. cruzi* by >95% and anti-IL-2R p55 mAb inhibits the response by 70%. The mRNA for cytokines IL-1ß, IL-2, IL-5, IL-6, IFN-γ, and TNF-α are detected by cDNA PCR after *T. cruzi* co-culture with PBMC, peaking on day 3. No IL-4 or IL-10 mRNA are detected. 85,000 U of IL-1 and 60,000 U of IL-6 are found in the supernatents *T. cruzi* stimulated PBMC by bioassay, but none from unstimulated PBMC. Monocytes, infected in the absence of apparent lymphocytes, assume activated morphology and accumulate mRNA for IL-1ß, TNF-α, and IL-6. T cells require accessory cells to proliferate and produce cytokine mRNA. A trypsin sensitive activity in lysates of *T. cruzi* stimulates lymphocyte proliferation with similar kinetics to whole *T. cruzi*. Thus, *T. cruzi* co-culture with human PBMC leads to lymphocyte proliferation, monocyte activation, and cytokine production. This *in vitro* model will be useful in the study of the immune aberrations that occur in the acute infection and the molecule(s) of *T. cruzi* that are involved in stimulating this response.

C 153 CLONING OF THE HYPOXANTHINE-GUANINE PHOSPHORIBOSYL TRANSFERASE GENE FROM *T. GONDII*, Geetha Vasanthakumar and Gregory E. Parish, Molecular Biology Section, Department of Biochemistry, Southern Research Institute, Birmingham, AL 35255.
Since hypoxanthine-guanine phosphoribosyl transferase (HGPRT) plays a key role in salvaging preformed purines for *T. gondii*, which 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 attempted to clone the gene encoding for *T. gondii* HGPRT and express it into *E. coli*. We report the cloning of the HGPRT cDNA by means of PCR using degenerate primers. Degenerate oligonucleotide primers based on conserved regions of the HGPRT amino acid sequence at phosphoribosylpyrophosphate binding site were used for PCR amplification of *T. gondii* cDNA. The PCR product of size ~500 bp was cloned into PCR1000 and sequenced. The sequence was compared with the available known HGPRT sequences. Southern blot analysis shows that this sequence is homologous to human, mouse, and *P. falciparum* HGPRT. Further isolation of the complete cDNA sequence and characterization of *T. gondii* HGPRT will be discussed.

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C 154 IDENTIFICATION OF SUBGROUPS OF *GIARDIA LAMBLIA* BASED UPON 18S RIBOSOMAL RNA SEQUENCE. Judith B. Weiss, Roche Diagnostics Research, Alameda CA 94501
Giardia lamblia, a parasitic intestinal protozoan, is an important cause of diarrheal disease worldwide. Speciation of the genus *Giardia* has been the subject of much research and controversy over the years. Recent studies have supported the concept of a single grouping or species of *Giardia* that is capable of being transmitted to humans and causing disease therein despite being present and viable in many mammalian hosts. A sensitive and specific PCR-based assay has been developed to detect and analyze G. *lamblia* DNA from fecal specimens. The oligonucleotide primers amplify a 182bp segment of the 18S ribosomal RNA gene. Efficient amplification required the inclusion of cosolvents (glycerol and dimethylsulfoxide) in the reaction. Following the optimization of conditions for amplification and hybridization of radiolabeled oligonucleotide probe with the amplified products, a sensitivity of less than one organism's worth of DNA was achieved. Thirty-five different G. *lamblia* strains obtained from various animal hosts and geographic locations were detected by this method. By more detailed analysis, the G. *lamblia* isolates could be divided into three subgroups based upon rRNA sequence. A substantial fraction of formalin-fixed fecal specimens obtained from patients suspected of having gastrointestinal parasites were shown to contain G. *lamblia* DNA sequences, including the "variant" sequences. These findings strongly corroborate the notion of a limited polymorphism and number of lineages within the species G. *lamblia*. Additional probes differentiated G. *muris* and G. *ardeae* isolates (the rRNA gene from these non-human *Giardia* species were amplified with the same primers).

C 155 IMMUNIZATION WITH *PLASMODIUM BERGHEI* SPOROZOITES INDUCES TH1 T CELL SUBSET. Katherine L. White and Urszula Krzych Department. of Immunology,Walter Reed Army Institute of Research, Washington, DC 20307 and Department. of Biology, The Catholic University of America Washington, DC 20064
Protective immunity against *P.berghei* malaria induced by immunization with irradiated sporozoites (SPZ) requires both humoral and cellular responses. Previously, we demonstrated that T cell proliferative reactivity to SPZ antigens does not correlate with protection against malaria. Balb/c (H-2^d) mice were protected following a single dose of 75,000 SPZ, but required two boosting immunizations of 20,000 SPZ to elicit anti-SPZ proliferative activity. The opposite was seen in C57Bl/6 (H-2^b) mice where the priming dose elicited SPZ specific T cell reactivity but two boosts were necessary to render protection. Protective immunity against malaria was difficult to achieve in C3H/HeN (H-2^k) mice, and anti-SPZ proliferative T cell responses were evident only after the removal of $CD8^+$ T cells. These observations suggested not only strain associated differences in response to SPZ antigens, but also that protective immunity may be multi-factorial involving functionally diverse T cell subsets. Therefore, in the present study we investigated the discordance between proliferative responses to SPZ antigens and protective immunity by evaluating T cell subset induction through lymphokine analysis of splenic cultures. The levels of IL-2 and IL-3 accompanied the proliferative reactivities in both Balb/c and C57Bl/6 mice, however, in C3H/HeN mice high levels of IL-3 were detected after the priming dose but decreased upon boosting, when IL-2 and proliferative reactivity was uncovered. IL-4 was not detected in any of the three strains following the priming or boost immunizations, although all strains produced specific anti-circumsporozoite (CS) IgG antibody. Levels of IL-6 that correlated with the levels of antibody were measured in the three strains suggesting that IL-6 may drive anti-CS antibody production. Finally IFNγ was present in unprimed mice and increased significantly in all strains upon boosting. Based on these findings we conclude that immunization with irradiated SPZ induces Th1 T cell subpopulation. The precise function of these T cells in anti-malaria immunity is currently under investigation.

C 156 AMPLIFICATION OF THE IMP DEHYDROGENASE GENE IN MYCOPHENOLIC ACID RESISTANT *L. DONOVANI* AND *T. BRUCEI*, Keith Wilson, Randy Berens[1], and Buddy Ullman, Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR, 97201 and [1]University of Colorado Health Sciences Center, Denver, CO, 80262 To analyze mechanisms by which parasitic protozoa develop resistance to drugs, strains of Leishmania donovani promastigotes and Trypanosome brucei procyclic forms were generated by their ability to proliferate in incrementing concentrations of mycophenolic acid (MPA), an inhibitor of IMP dehydrogenase (IMPDH) activity. Genes encoding the *L. donovani* and *T. brucei* IMPDH proteins were isolated and sequenced from genomic libraries and exploited to evaluate drug resistance mechanisms in the MPA-resistant strains. Southern blot analysis demonstrated that both MPA-resistant cell lines have amplified their IMPDH gene copy number 10 - 15 fold over that of parental strains. The MPA-resistant *L. donovani* cell line also expressed augmented amounts of IMPDH transcripts. Separation of chromosomes by pulsed field gel electrophoresis revealed that the amplified copies of the IMPDH gene in the MPA-resistant *L. donovani* could be localized to a linear extrachromosomal element of 280 kb. This multicopy extrachromosomal DNA, as well as the drug resistance phenotype, is stable in the absence of selective pressure for over 18 months. These data support a novel amplification of a linear DNA element in drug-resistant Leishmania and provide the first evidence for gene amplification events in Trypanosoma.

C 157 CELLULAR IMMUNE RESPONSES IN HUMAN LYMPHATIC FILARIASIS, Maria Yazdanbakhsh, Yvonne C. Kruize, William Paxton, Erliyani Sartono, Murray E. Selkirk, Rick M. Maizels and Felix Partono, Department of Parasitology, University of Leiden, The Netherlands. Depatment of Biochemistry and Biology, Imperial College, UK. Department of Parasitology, University of Indonesia, Jakarta, Indonesia.
Residents of 8 villages in an area endemic for Brugian lymphatic filariasis in Sumatra, Indonesia were examined parasitologically and clinically for signs of infection. Peripheral blood was obtained from 170 volunteers. Cellular and humoral responses were characterized in these individuals who were also typed for HLA class I and II. The antibody IgG4 responses to Brugia malayi antigens were elevated in individuals harbouring active infection. T cell proliferative responses to adult Brugia malayi extracts were significantly higher in individuals with high IgG4 compared to people with low anti-Brugia IgG4 antibodies. In addition, individuals with high anti parasite IgE showed increased T cell proliferative indices. Seventy five percent of the latter group was comprised of elephantiasis patients. The release of IFNγ and IL4 were measured in supernatants of peripheral blood mononuclear cells stimulated with anti-CD2 and rIL2. It was apparent that there was a parasite density-dependent inhibition of IFNγ producing cells and expansion of IL4 secreting cells. This was confirmed in experiments where T cell lines and clones were generated to total parasite extract as well as recombinant antigens. T cell lines generated from individuals harbouring light infection released higher amounts of IFNγ compared to people with heavy infections. In a cloning experiment, we found that 90% of the T cell clones had a cytokine secretion pattern consistent with TH2-type cells, 10% with TH0-type pattern and none with TH1 pattern. The HLA analysis revealed a link between class II DQ and DR loci and pathology in our study population. There was a significant increase in an epitope present on DQW1, 8 and 9 antigens in individuals protected from developing pathology. There was also an increase in frequency of DRW13 in patients that mounted a high IgE responses to parasite antigens. This is of particular interst with respect to pathology.

Gene Regulation and RNA Metabolism

C 200 IDENTIFICATION OF POSSIBLE TRANSCRIPTION PROMOTERS IN TRYPANOSOMA CRUZI
Ajioka, J.Y., Hariharan, S. and Swindle, J.; Dept. of Microbiology and Immunology, University of Tennessee, 858 Madison Ave. Memphis, TN 38163.

Transient and stable transformation systems have been developed for Trypanosoma cruzi. Both systems have been used to study the expression of the calmodulin-ubiquitin associated (CUB) and ubiquitin-fusion (FUS) genes. The CUB2.65 and FUS1 genes are tandemly arrayed and separated by an intergenic sequence of approximately 400bp. Expression of the chloramphenicol acetyl transferase (CAT) gene from the potential CUB2.65 promoter is dependent on both the potential promoter sequence and a proper T. cruzi 3' sequence. Similarly, the possible FUS1 promoter is capable of driving CAT gene expression in the transient transfection system. To determine if the possible FUS1 promoter identified in the transient system functions on the chromosome, tandem gene replacement experiments were carried out. Stable transformants of T. cruzi have been isolated in which the tandemly arrayed CUB2.65 and FUS1 genes have been simultaneously replaced by the neomycin phosphotransferase (NPTII) and CAT genes respectively. The regulation of expression of the NPTII and CAT genes during the developmental cycle is being investigated and will be compared with that of the CUB2.65 and FUS genes.

C 201 DIFFERENTIAL SPLICING EFFICIENCY OF GROUP I INTRONS SITUATED IN THE 16S-LIKE RIBOSOMAL RNA GENES OF TWO *PNEUMOCYSTIS CARINII* ISOLATES, J.W. Anderson and P.A. Liberator, Department of Animal Biochemistry and Molecular Biology, Merck Sharp & Dohme Research Laboratories, Rahway, N.J. 07065

Steroid-induced immunosuppression of rodents initiates the expansion of a latent *Pneumocystis carinii* (P.c.) infection in the lung. The 16S-like ribosomal RNA (rRNA) genes from P.c. prepared from both immunosuppressed rats and mice have been cloned using the polymerase chain reaction (PCR). Nucleotide sequence of multiple clones indicates that the rRNA genes from the two isolates are not identical to one another. Each of the clones and each of the rRNA genes in the respective genomes does however contain a characteristic group I intervening sequence (IVS). The group I IVS' are nearly 400nt in length and are similarly positioned very close to the 3'-end of the gene.

IVS' from both isolates undergo accurate and efficient autocatalytic splicing *in vitro* without protein assistance or energy consumption. Recombinant plasmids containing the respective introns have been constructed so as to be able to also assess splicing activity in bacteria. Specifically, accurate splicing of the intron from the construct reconstitutes beta-galactosidase activity. Splicing of both introns does occur in bacteria and is orientation dependent. The mouse-derived *P. carinii* intron splices much more efficiently than the rat-derived counterpart. Site-directed mutagenesis indicates that a "G" residue at nucleotide position -1 from the 5'-end of the intron is an undesirable substitution. The splicing efficiency of the rat-derived intron in plasmids containing nucleotides "A" or "C" in addition to the wild type "U" at this position is equivalent. Conversely, a "U" at position -1 in the mouse-derived intron is clearly the most efficient representative. Curiously, only two group I introns in a recent compilation of 87 had nucleotides other than a "U" at this position.

C 202 USE OF PCR AMPLIFICATION OF TRYPANOSOMA CRUZI MINICIRCLE DNA FOR DIAGNO OF CHRONIC CHAGAS DISEASE, Herbert Avila[1], Ot: Thiemann[1], Wim Degrave[3], Jose Borges[4], Carlos Morel[3] and Larry Simpson[2], [1]Department of Biology and [2]Molecular Biology Institute, University of California Los Angeles, CA 90025. [3]Department of Biochemistry and Molecular Biology, [4]Department of Tropical Medicine, FIOCRUZ, Rio de Janeiro, Brasil.

A variety of biological specimens, including whole blood, tissue biopsies and fecal material, can be stored as a lysate in 3M guanidine HCl/0.1M EDTA (GE). DNA stored in these lysates remains undegraded for 1 month at 37°C. Human, animal and insect specimens have been stored in GE buffer and analyzed for the presence of *Trypanosoma cruzi*. *T. cruzi* kinetoplast kDNA in GE lysates can be cleaved using the chemical nuclease, 1,10 phenanthroline-copper ion. This procedure liberates linearized minicircle molecules from network catenation, distributing them throughout the lysate, and allowing a small aliquot of the original lysate to be analyzed by PCR amplification of minicircle-specific sequences. This increases the sensitivity of the method dramatically for the detection of small number of trypanosomes in a large volume of blood. A panel of over 100 blood samples from chronic chagasic patients and healthy individuals, was screened using the GE/PCR method. The test showed a sensitivity of 100% and a specificity of 90% as compared to serological tests. In addition, the GE/PCR method proved to be twice as sensitive as xenodiagnosis. This PCR-based test should prove useful as a replacement and/or complement for xenodiagnosis and serology in clinical and epidemiological studies of chronic Chagas' disease.

C 203 CHARACTERISATION OF THE TRYPANOSOMA BRUCEI gGAPDH PROMOTER, Norbert Bakalara, Gilles Kendall, Fred Opperdoes, ICP-TROP. 74/39, Universite catholique de louvain, Bruxelles 1200.

Regions 5' of the glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) gene from Trypanosoma brucei were tested for their ability to promote chloramphenicol acetyl-transferase (CAT) expression on introduction by electroporation into the parasite. Deletion analysis mapped the gGAPDH promoter to within 403 nts of the start of transcription . A transcription initiation site was mapped at approximately -190 nts from the ATG start codon by RNaseA protection and by primer extension. Two additional transcription initiation sites were mapped by protection experiments at positions -282 and -352 nts. The higher expression of gGAPDH in bloodstream T.brucei, compared to procyclic (insect) forms, was largely attributed to differences in promoter activity. The glycosomal promoter gave rise to relatively high CAT signals upon transfection into bloodstream form and relatively low signals in procyclic, compared with levels resulting from transfection with the procyclin acidic repetitive protein (PARP) promoter. In addition, RNase protection data showed a higher level of gGAPDH primary transcripts in bloodstream T brucei. The PARP mini-exon addition region abolished transient CAT expression directed by either the gGAPDH or PARP promoters in bloodstream T.brucei implying that trans-splicing can be a point of stage-specific gene regulation.

The gGAPDH promoter was also able to promote CAT activity in Leshmania mexicana and Crithidia lucillae.

C 204 KINETOPLAST GUIDE RNAS ARE ASSOCIATED WITH A 13S COMPLEX WHICH CONTAINS TERMINAL URIDYLYL TRANSFERASE AND SEVERAL OTHER PROTEINS, Andreas Bakker, Agda M. Simpson and Larry Simpson, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

The mitochondrial TUTase activity in Leishmania tarentolae separates in gel filtration and sedimentation analysis into three fractions: 30S, 13S (330 kd), and 8S. The 30S fraction probably represents nonspecific binding to mitochondrial ribosomes. The leading edge of the 13S peak contains all the mitochondrial gRNAs. The endogeneous bound gRNA is a substrate for the 13S TUTase activity. UV crosslinking and gel retardation experiments showed that 6-8 proteins of this complex interact with the U-labeled 3' termini of the gRNA. Incubation of the endogeneous gRNA-labeled 13S complex with heparin decreases the size of the labeled complex to 8S, possibly by releasing several proteins. We suggest that the 13S TUTase-gRNA complex represents a component of the enzymatic machinery involved in RNA editing of maxicircle transcripts.

C 205 HEAT-SHOCK REGULATES THE POOL SIZE OF THE SURFACE ZINC-PROTEINASE (GP63) TRANSCRIPTS OF *LEISHMANIA* PROMASTIGOTES, Chaudhuri, Gautam, Division of Biomedical Sciences, Meharry Medical College, Nashville, TN 37208.

The major surface glycoprotein, gp63, of the parasitic protozoan, *Leishmania* is a unique zinc-proteinase with potential roles in parasite uptake by macrophages, in digestion of medium proteins for nutritional purposes and in the evasion of the cytolytic action of the phagolysosomal hydrolases by the parasite inside the macrophages. Heat-shock is associated with the developmental changes of the parasite from promastigotes to amastigotes. Amastigotes of *L. mexicana amazonensis* apparently have 2-5 fold higher concentration of gp63 mRNA than that of the promastigotes. These amastigotes were isolated from the infected cultured J774G8 macrophage-like cells which were grown at 30-33°C. When the cultured promastigotes, which grow in axenic culture medium at 25°C, were heat-shocked at 30°C for 2 h, the steady-state concentration of gp63 mRNA was increased 2-3 folds. Increase in the temperature of heat-shock to 37°C caused 10-20 fold decrease in that mRNA level. Similar effects of heat-shock was also observed with *L. donovani* promastigotes. The negative effect of heat-shock at 37°C on the pool size of gp63 mRNA may not be due to impairment of the processing of the pre-mRNA. No high molecular size gp63-specific RNA was found to be accumulated in heat-shocked cells. Although, the cells heat-shocked at 37°C for 2 h grew normally at 25°C afterwards, the gp63 mRNA pool size was not regained within subsequent 4 h incubation at 25°C. The pool size became normal after 16-20 h post-heat-shock incubation at 25°C. Incubation of the promastigotes at acidic pH (pH 4-5) or under hypoxic conditions also led to decrease in the pool size of gp63 mRNA. Heat-shock of the promastigotes at 37°C under those insults accelarated the decrease of this pool. Alterations in the steady state concentration of a potential virulent factor mRNA in *Leishmania* under several insults that the cells normally experience during their usual developmental process, suggests the possibility of similar regulations in the expression of this parasite surface metalloproteinase gene under natural conditions. Supported by National Science Foundation Minority Research Center for Excellence in Cell and Molecular Biology Grant # R118714805.

C 206 CR3 AND CR4 ARE HIGHLY EDITED CRYPTOGENES THAT ARE EDITED IN DIFFERENT DEVELOPMENTAL STAGES OF *TRYPANOSOMA BRUCEI*

Robert A. Corell, Peter J. Myler, and Kenneth D. Stuart, Seattle Biomedical Research Institute, Seattle, WA 98109-1651

kRNA editing is a post-transcriptional process that inserts and more rarely deletes uridines from primary kinetoplast transcripts. In the most highly edited genes over 50% of the final transcript is created by editing. These genes have the general characteristic of being GC rich with a strong G vs. C strand bias. We have investigated the transcripts from two such regions of the *T. brucei* kDNA, CR3 and CR4. CR3 and CR4 are transcribed into highly edited, polyadenylated, mRNAs. Although CR3 is abundantly transcribed in both bloodstream and procyclic life cycle stages, CR3 is edited to only a limited degree in the procyclic stage, while it is extensively edited in the bloodstream stage. In addition the editing which does occur in the procyclic stage differs substantially from that in the bloodstream stage. CR4 on the other hand is extensively edited in the procyclic stage. We have isolated several CR4 cDNAs which may represent truncated 5' molecules, predicted by the transesterification model. These molecules are unedited at their 5' ends but partially edited at their 3' ends, and many of the molecules end in runs of uridines. The results suggest that editing does not occur in a strictly 3' to 5' direction.

C 207 **ANTIGENIC VARIATION OF A MAJOR MEROZOITE SURFACE MOLECULE IN THEILERIA ANNULATA, Joanne Dickson and Brian R. Shiels.** Wellcome Unit of Molecular Parasitology, Glasgow University, Bearsden Road, Glasgow, Scotland, U.K.

Monoclonal antibody 5E1 recognises a 30kDa major polypeptide on the surface of merozoites (J.Glascodine et al '90). Serum from animals immune to the Ankara strain of Theileria annulata also recognises a 30kDa molecule and immunoprecipitation experiments indicated that this molecule is identical to that recognised by 5E1.

Analysis by Western blotting, of merozoites from cloned cell lines of Theileria annulata (Ankara) showed that the 30kDa molecule is polymorphic. This was characterised by a shift in the molecular mass of the 30kDa molecule to 32kDa.

In addition, 2-D gel electrophoresis, Western blotting and peptide mapping of the antigen has shown that the two forms of the molecule are closely related but are likely to have differences in polypeptide sequence.

Furthermore, stocks of piroplasm from different geographical regions can be distinguished on Western blots on the basis of the polymorphism of this antigen.

These results implicate this molecule as a potential candidate for use as a component of a sub-unit vaccine, and as a marker for stock characterisation.

C 208 MECHANISM OF SUPER-INDUCTION OF PROCYCLIN (PARP) RNA, Patricia L. Dorn and John C. Boothroyd, Department of Microbiology and Immunology, Stanford School of Medicine, Stanford CA. 94305-5402

Procyclin RNA, which encodes the major surface protein of the insect form trypanosome, is rapidly and abundantly induced during differentiation from the mammalian bloodstream form to the insect form trypanosome. Expression of procyclin RNA appears to be under negative control in the bloodstream form trypanosome, mediated by a labile protein, since treatment with a variety of protein synthesis inhibitors results in super-induction of procyclin RNA even in the absence of signals that trigger differentiation. The mechanism of this super-induction does not appear to be at the level of transcription initiation since no differences in the amount of procyclin RNA were observed in nuclear run-on assays under conditions which showed super-induction of procyclin RNA by Northern blot analysis. Other mechanisms for the super-induction such as stability of the message are being investigated and will be discussed.

C 209 Identification of a Neutral Surface Metalloproteinase on 13 species of *Leishmania* promastigotes isolated from humans, *Crithidia fasciculata*, and *Herpetomonas samuelpessoai*. Robert Etges, Institut de Biochimie, Université de Lausanne, Ch. des Boveresses 155, CH-1066 Epalinges, Switzerland

Promastigotes of thirteen species of *Leishmania* isolated from human patients, as well as *Leishmania enriettii*, *Crithidia fasciculata* and *Herpetomonas samuelpessoai*, were examined for the expression of an amphiphilic, surface-oriented neutral metalloproteinase by surface radioiodination of living cells, fractionation by Triton X-114 extraction and phase separation, and zymogram analysis by fibrinogen-SDS-PAGE. In all species of *Leishmania*, and the two monoxenous trypanosomatid parasites of insects, an ectoproteinase similar to the Promastigote Surface Protease, or PSP ("gp63"), was observed. In contrast, neither *Phytomonas* sp. nor "*Leishmania tarentolae*" (reclassified as *Trypanosoma platydactyli*) express a detectable surface metalloproteinase. The presence of the functionally conserved metalloproteinase at the surface of *Crithidia* and *Herpetomonas*, which never encounter a mammalian macrophage during the course of their life cycles, suggest the the enzyme may not be involved in the infection of the mammalian host by *Leishmania*, but rather contributes to the survival of the protozoan in the environment of the insect midgut. These findings call into question the vast amount of attention devoted to the promotion of PSP as a vaccine component to protect the mammalian host from infection.

C 210 DNA POLYMORPHISMS IN FILARIAL SUSCEPTIBLE AND REFRACTORY STRAINS OF *AEDES AEGYPTI* USING RAPD-PCR, S.W. Gordon, S.E. Brown, R.B. Grieve, and D.L. Knudson, Departments of Microbiology, Entomology, and Pathology, Colorado State University, Fort Collins, CO 80523

The random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) is based upon the amplification of genomic DNA using a single primer, 10 nt of arbitrary sequence. These primers detect polymorphisms in the absence of specific genomic nucleotide sequence information. The polymorphisms function as genetic markers and can be used to construct genetic maps. The RAPD-PCR technique has been applied to detecting DNA polymorphisms in 4 strains of *Aedes aegypti*, which are susceptible (Liverpool and 7D) and refractory (Rexville D and Liverpool R) to infection with *Dirofilaria immitis*. Forty-two primers were screened using genomic DNA from individual mosquitoes of the four strains. Genotype specific patterns of bands ranging in size from 0.5 to 2.0 Kbp were observed in ethidium bromide stained 1.4% agarose gels. Nine primers distinguished all four strains. Banding patterns were inherited equally in F1 offspring of a cross between susceptible and refractory parents. The utility of these primers for genome mapping will be discussed.(Supported by the MacArthur Foundation)

C 211 THE CHARACTERIZATION OF MITOCHONDRIAL RIBONUCLEOPROTEIN (RNP) COMPLEXES INVOLVING GRNAS IN *TRYPANOSOMA BRUCEI*.
H. Ulrich Göringer, Donna J. Koslowsky, Tony H. Morales and Kenneth Stuart, Seattle Biomedical Research Institute, Seattle, WA 98109-1651

The expression of a number of mitochondrial genes in the parasitic protozoa *Trypanosoma*, *Leishmania* and *Crithidia* requires a post-transcriptional RNA-processing event termed RNA editing. During editing pre-mRNA molecules are converted into translatable messages by the insertion and deletion of multiple uridine residues. A key component of the mitochondrial machinery that provides at least part of the information for editing, is a new class of small, stable RNA molecules, so-called guide RNAs (gRNAs). These molecules are complementary to edited domains of the mature mRNAs and contain oligo(U) tails at their 3'-ends. The present paradigm for the mechanism of editing requires a gRNA/pre-mRNA hybrid to perform a set of subsequent transesterification reactions with the terminal U residue of the gRNA serving as the nucleophile. A nearly ubiquitous feature of RNA molecules involved in a plethora of complex biological events is their association with proteins to form functionally active RNP particles. By using a gel mobility-shift assay we were able to identify four different mitochondrial RNP complexes involving gRNA molecules under *in vitro* conditions. The formation of the complexes is dependent on the input of mitochondrial lysate in a manner that correlates with theoretical binding curves. We present the kinetics of complex formation, temperature and ATP requirements as well as monovalent and divalent ion dependencies.Label transfer experiments identify a minimum of five different protein components as direct binding partners in the complex formation. The data provide first evidence for an "editosome" RNP particle and are suggestive of a sequential assembly of the editing machinery.

C 212 DEVELOPMENTAL STAGE-SPECIFIC TRANSCRIPTION OF TWO METACYCLIC VSG GENE EXPRESSION LOCI IN TRYPANOSOMA BRUCEI, Sheila V. Graham, Keith R. Matthews, Paul G. Shiels and J. David Barry, Wellcome Unit of Molecular Parasitology, Institute of Genetics, University of Glasgow, Glasgow, G11 5JS, Scotland, U.K.

African trypanosomes first express the variant surface glycoprotein (VSG) coat at the metacyclic stage in the tsetse fly using a system very different from that used in bloodstream trypanosomes. Only a small, specific subset (<=27, 1-2%) of VSG genes are expressed at the metacyclic stage. We have shown that metacyclic VSG genes occupy structurally distinct telomeres and have a stage-specific activation mechanism: they are activated in situ in the fly. There are a number of distinctive features of these telomeres - they contain very short 70 bp repeat regions; they comprise low copy number sequence; they incorporate very short transcription units containing only the VSG gene. We have found that two metacyclic VSG genes are transcribed stage-specifically. While promoters for bloodstream VSG genes are active throughout the parasite life cycle, the metacyclic VSG gene promoters appear to be active only at the metacyclic stage. Because absolute transcriptional control has not yet been observed for any other trypanosome gene, we are undertaking a detailed study of the promoter regions. We have mapped and sequenced transcription initiation sites and are testing putative promoter regions in vivo.

C 213 RNA EDITING: IDENTIFICATION OF A PRE-EDITED mRNA SPECIFIC ENDORIBONUCLEASE AND ITS ROLE IN FORMATION OF CHIMERIC GUIDE RNA/mRNA MOLECULES *IN VITRO*, Stephen L. Hajduk, Victoria W. Pollard and Michael E. Harris, Deptartment of Biochemistry, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, AL 35294

RNA editing of trypanosome mitochondrial mRNAs results in the addition of uridines not encoded in the DNA which alters the protein coding information. We have examined this novel RNA processing event *in vitro*. We have chosen to examine in detail the edited region located at the 5' end of cytochrome b (CYb) mRNA where the added U's create an AUG initiation codon for this mRNA. Incubation of synthetic pre-edited CYb mRNA with trypanosome mitochondrial extracts results in endoribonuclease cleavage at the 3' portion of the pre-edited region, where editing is thought to commence. The edited version of CYb RNA is not cleaved. Addition of synthetic gRNA to the reaction results in the formation of a chimeric molecule containing the gRNA and the 3' cleavage product of the CYb substrate RNA. In addition, uridines from the synthetic gRNA appear to be transfered to the chimeric product and to the CYb substrate RNA.

C 214 **THEILERIA ANNULATA MEROZOITE ANTIGEN GENE, Khalid Hussain, Susan McKellar, Norma Buchanan, Andrew Tait and Brian Shiels,** Wellcome Unit of Molecular Parasitology, Glasgow University, Scotland, U.K.

Tropical theileriosis is a disease of cattle caused by the tick-borne protozoan parasite, Theileria annulata. The two invasive stages are potential targets for a protective immune response and thus molecules from these stages could be possible components of a sub-unit vaccine. We have cloned several copies of a merozoite surface antigen gene (mag) which show stage specific expression. Sequence analysis of a 3kb fragment of mag shows three distinct regions: a 5′ unique sequence, a bank of direct repeats and a 3′ unique sequence. The bank of repeats 1243bp in length and is made up of 3 different types of repeats: a 14bp repeat, a 73pb repeat (Z) and a 76bp repeat (K). Each Z and K repeat is enclosed at the 5′ and the 3′ ends by a 14bp repeat. The repeats are manifested at the protein sequence level and represent the greatest cell surface probability. At the point of termination of the repeats the sequence is virtually homologous to the E.coli chi sequence, hence implicating a possible recombination event at the site. A summary of Southern hybridisation using mag as a probe is as follows: (i) piroplasm genomic DNA digested with EcoRI shows a complex pattern with a low level of variability between stocks. (ii) Cloned macroschizont cell line genomic DNA digested with EcoRI shows segregation of the parental pattern, with at least two copies per clone. (iii) PFGE of a cloned macroschizont cell line shows that the 2 copies reside on 2 different chromosomes. (iv) In a piroplasm genomic DNA digest with EcoRI sequences are revealed which only hybridise with (a) 5′ unique sequence, (b) 5′ and 3′ unique sequences and (c) 3′ and repeat sequences. These results will be discussed.

C 215 BACTERIAL EXPRESSION, PURIFICATION AND IMMUNOGENICITY OF THE *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE ANTIGEN, MSA-2

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Studies on a glycoprotein expressed on the merozoite surface of *Plasmodium falciparum* have shown that it could be used as a component antigen in a vaccine against malaria. This protein, termed MSA-2, has been characterized from several different isolates and has an isolate-specific M_r that ranges in size from 35 - 50 kDa. Comparison of the MSA-2 molecules from the isolates so far described, reveals highly conserved amino and carboxy terminal sequences flanking a variable region which includes both repetitive and non-repetitive sequences The molecules characterized from all isolates can be broadly grouped into two allelic families.

The majority of the coding sequence of the MSA-2 gene isolated from a cloned line of *P. falciparum* (3D7) was expressed in a bacterial expression system. The recombinant protein was expressed as a soluble protein in which six histidine molecules were appended to the amino terminus of the protein. This facilitated purification away from bacterial contaminants using metal affinity chromatography. Further purification was achieved using anion exchange chromatography followed by reverse phase HPLC. This resulted in the high purity required for clinical testing.

The immunogenicity of purified recombinant MSA-2 was tested in several strains of mice and in rabbits. Responses were measured by ELISA against peptides or various recombinant MSA-2 proteins and by immunofluorescence on fixed smears of parasitised red blood cells. Immune responses varied depending on the species and strain of mouse. High antibody titres were measured in sera from H-2^k and outbred mice. Responses to both variable and conserved regions of the molecule were detected although these varied depending on the strain of mouse. Antibody responses in rabbits were generally low.

C 216 CROSS-SPECIES PROTECTION WITH A PURE PROTEIN FROM LEISHMANIA DONOVANI, Charles L. Jaffe and Nurit Rachamim, Dept. Membrane Research & Biophysics-MacArthur Center for Tropical Medicine, Rehovot, Israel.

Preliminary studies showed that a pure protein, dp72, from L.donovani could reduce liver parasite burden in BALB/c mice (Jaffe etal, 1990). The mechanism of this protection was examined. Mice immunized with dp72 (5μg) and C. parvum produce high antibody titers, >1/500, to this protein. However, the parasite burden, measured by both limiting dilution assay, and tissue biopsy, at 1 day post-infection (p.i.) with 10^7 promastigotes (Khartoum) was similar in both the control and immunized mice. This suggests that the protection is not due to antibody mediated killing immediately following infection. By 20 days p.i. the liver parasite burdens were 60% lower in the immunized mice and by 108 days p.i. the burden was further reduced to 78% of the control mice. Antigen specific T-cell proliferation to dp72 in vitro was abolished with either anti-CD4 or anti-CD4 and -CD8 antibodies. However, treatment with anti-CD8 antibodies, alone, enhanced antigen specific proliferation to dp72. Depletion in vivo of either CD4 or CD8 T-cells abolished protection, suggesting that both T-cell subsets are important in preventing disease. Passive transfer of antibodies to IFN-gamma also prevented the protection of immunized mice. A 60 kDa antigen which cross-reacts with monoclonal antibodies to dp72 is present in L.major, L.aethiopica and L.tropica. Lymphocytes from mice immunized with dp72 also proliferated when stimulated with total freeze/thaw homogenates from L.donovani, L.major, Leptomonas and Crithidia. No proliferation to other pure antigens such as gp63 or lipophosphoglycan from L.major, or gp70-2 from L.donovani was observed. The ability of dp72 from L.donovani to protect immunized BALB/c mice against L.major which causes cutaneous leishmaniasis was examined. Lesions were apparent in the control mice 34 or 50 days after challenge with 10^6 or 10^4 L.major promastigotes (Fredlin), respectively. Even after 90 days no leisons were evident in the immunized mice. This is the first report of a pure protein which protects against Leishmania species causing different diseases. Jaffe CL, etal. (1990) J. Immunol. 144: 699. Supported by the UNDP/World Bank/WHO TDR Programme, the John and Catherine T. MacArthur Foundation and the U.S. - Israel A.I.D. Programme.

C 217 DEVELOPMENT OF AN *IN VITRO* RNA EDITING ASSAY IN *TRYPANOSOMA BRUCEI*.

Donna J. Koslowsky, Tony H. Morales and Kenneth Stuart, Seattle Biomedical Research Institute, Seattle, WA 98109-1651

We have developed an assay system to monitor the association of specific mitochondrial proteins and RNAs on exogenous messages. Following incubation in mitochondrial lysate, ^{32}P-labeled pre-edited mRNA transcripts assemble into several stable ribonucleoprotein particles which can be detected using gel electrophoresis in low percentage native polyacrylamide gels due to their retarded mobility. The protein association requires Mg^{+2}, and is transcript-specific as demonstrated by competition with homologous RNA (and not heterologous RNA). Complex formation shows distinct temperature optima for two different mRNAs tested. ATPase 6, which is edited in both life forms, has a temperature optimum at 37°C, while CYb, which is predominately edited only in procyclic forms, has a temperature optimum at 27°C. Using conditions which show optimal RNP formation, we have detected gRNA/mRNA chimeric molecules, where the gRNAs are covalently linked to the exogenously added mRNA transcripts. These molecules provide the first evidence for *in vitro* trans-esterification of gRNAs to mRNAs. The development of this assay system will allow us to characterize the factors involved in RNA editing in *T. brucei*.

C 218 IMMUNIZATION WITH IRRADIATED *Plasmodium falciparum* SPOROZOITES INDUCES T CELLS SPECIFIC FOR SPOROZOITE-, LIVER- AND BLOOD-STAGE ANTIGENS. *Urszula Krzych, Jeffrey Lyon, Teresa Jareed, Matthew Seguin, Michael* Hollingdale, Carmen Rodriguez, and W. Ripley Ballou. Dept. of Immunology, WRAIR, Washington, DC 20307 and BRI, Bethesda, MD 20850

Immunization with irradiation-attenuated Plasmodia sporozoites induces protective immunity characterized by both humoral and cellular responses. Although the majority of antibody responses are directed against the circumsporozoite (CS) protein antigen, it remains unknown whether T cell involved in protective immunity are directed against determinants on the CS protein or against other antigens associated with the preerythrocytic or erythrocytic stages of malaria. Using *P. falciparum*-immune peripheral blood lymphocytes obtained from volunteers vaccinated with irradiated sporozoites, we analyzed T cell responses against a panel of antigens representing the sporozoite, liver and blood stage antigens. The proliferative and cytolytic (^{51}Cr release) analyses identified T cells recognizing parasite antigens, antigens expressed by live vectors or as recombinant proteins or synthetic peptides. Responses were analyzed at different times during immunization, including pre-immunization, pre- and post-challenge. The results demonstrate the presence of T cells with specificities not only to the sporozoite stage antigens, but also to antigens representing the other two stages of malaria. Therefore, these studies suggest that protective mechanisms induced by irradiated sporozoities might be generated during coexpression of the different stage antigens by the liver schizonts. The implications of these findings for vaccine development will be addressed.

C 219 DIVERSITY IN POLYMORPHIC ANTIGENS OF PLASMODIUM FALCIPARUM IN FIELD ISOLATES FROM KENYA, Susan Kyes, Kevin Marsh and Chris I. Newbold, Molecular Parasitology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK

Antigenic diversity is thought to allow pathogens the ability to evade host immune recognition. We are studying diversity in field isolates of *Plasmodium falciparum* by a comparison of *three single-copy genes encoding polymorphic proteins.* Genes for S antigen (SAg), merozoite surface protein 1 (MSP-1) and merozoite surface protein 2 (MSP-2) are characterized by blocks of non-conserved tandem repeats, flanked by conserved or semi-conserved regions. Polymerase chain reaction (PCR) was used to amplify tandem repeat regions of DNA prepared from blood samples. PCR product size variation was found for each gene. Multiple PCR products from single DNA samples may be indicative of multiple infections; this was supported by cloning and sequencing of different-sized MSP-1 products. Initial sequence data show that MSP-1 and MSP-2 polymorphisms are consistent with previously published allele sequences. In contrast, SAg has proved difficult to amplify in quantity sufficient for sequencing, possibly due to the large number of repeats (20-100 repeats in SAg, as opposed to 2-20 repeats in MSP-1 and MSP-2). Application of this type of analysis to field samples collected with regard to time and geographical location will help describe development and maintenance of diversity in *P. falciparum*.

C 220 HSP70 GENES IN SCHISTOSOMA MANSONI: REGULATION BY STRESS AND BY DEVELOPMENTAL PROGRAM. R. Levy-Holtzman, S. Neumann and I. Schechter. Dept. Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

The cDNA and genes encoding hsp70 were cloned from adult worms and sequenced. Northern blots and other studies demonstrate that hsp70 gene(s) are regulated by two mechanism. Stress induction is specific to hsp70 and refers to transient and high level of hsp70 transcription during cercaria - schistosomula transformation, and after heat shock (42°C) of adult worms. Developmental program common to hsp70 as well as to paramyosin, myosin and other genes, refers to constitutive transcription at certain developmental stages (miracidia$^+$, sporocyst$^+$, cercaria $^-$, adult worm$^+$), and to the termination of transcription during sporocyst - cercaria transformation. The latter findings raise the possibility that the coordinated expression of large sets of different genes during schistosome metamorphosis may be regulated by similar 5' DNA consensus sequence(s). Analyses of several hsp70 genes reveal two types of non-functional genes and one functional gene. The promoter of the functional gene contains two heat shock responsive elements (HSE) that differ from the consensus sequence, CnnGAAnnTTCnnG, by one (HSEI) or three (HSEII) base changes. Synthetic ^{32}P-labeled HSEI and HSEII reacted with schistosome extracts were analyzed by the gel retardation assay to investigate heat shock transcription factors (HSTF) in relation to the pattern of hsp70 gene expression. Active factors that can bind HSEI or HSEII were not found in adult worms in which hsp70 mRNA is constitutively expressed. However, adult worms exposed to heat shock (42°C) and young schistosomula (4 hr after transformation) contain (putative) active HSTF binding with HSEI, but not with HSEII. The HSEI-HSTF interaction is specific because it is inhibited by cold HSEI and not by HSEII or other DNA fragments. We tentatively conclude that HSEI plays a major role in the regulation of hsp70 by stress, and that other 5' DNA elements are involved in hsp70 regulation by the developmental program. These issues and functional evaluation of the parasite hsp70 promoter in eukaryotic cell lines, are studied.

C 221 QUANTITATIVE AND SPECIES-SPECIFIC EVALUATION OF INFECTIVITY FOR SEVEN CHICKEN *EIMERIA* SPECIES, P.A. Liberator, J.W. Anderson, P.R. Chakraborty, M. Dashkevicz, S.D. Feighner and H. Profous-Juchelka, Department of Animal Biochemistry and Molecular Biology, Merck Sharp & Dohme Research Laboratories, Rahway, N.J. 07065.

Infection by protozoan parasites of the genus *Eimeria* causes coccidiosis, a disease which is a major problem to the poultry industry. Trickle infection with any of the seven species of chicken *Eimeria* leads to protection against reinfection, but there is no cross-protective immunity between different species. Accordingly, an efficacious live vaccine to protect against coccidiosis in the field must be composed of viable organisms from each of seven species. We have developed an assay to predict, in species-specific fashion, the relative viability of *Eimeria* spp. in chickens which have received a low level mixed infection. The ability to detect parasite in the intestinal epithelia and mucosa of vaccinated birds, the target tissue for these protozoa, verifies that the organisms are capable of penetrating the intestinal epithelium and initiating intracellular development. Nucleic acids are prepared from these parasitized host tissues and used as a substrate in the polymerase chain reaction (PCR). The PCR products are immobilized on a nylon membrane and serve as a target for species-specific radiolabeled oligonucleotide hybridization probes. Quantitative estimates of infectivity for each species are generated by scanning the membrane and relating the output to the hybridization signal from a titration of amplification standards. The assay is sufficiently sensitive to detect an infection resulting from as few as 100 oocysts. At this level of infection, the parasite component of the intestinal scraping is considerably less than 1% of the total material.

C 222 AN INTERGENIC G-RICH REGION IN KINETOPLAST MAXICIRCLE DNA REPRESENTS A PAN-EDITED CRYPTOGENE ENCODING S12 RIBOSOMAL PROTEIN, Dmitri A. Maslov, Nancy R. Sturm, Betina M. Niner, Eileen S. Gruszynski, Marian Peris and Larry Simpson, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

Six short G-rich intergenic regions in the maxicircle of Leishmania tarentolae are conserved in location and polarity in two other kinetoplastid species. We show that the G6 region represents a pan-edited cryptogene which contains at least two domains edited independently in a 3' to 5' manner connected by short unedited sequences. In the mature edited RNA, 117 uridines are added at 49 sites and 32 uridines are deleted at 13 sites, creating a translated 85 amino acid polypeptide. Similar polypeptides are probably encoded by pan-edited G6 transcripts in two other species. A minicircle-encoded gRNA overlaps 12 editing sites in G6 mRNA, and chimeric gRNA/mRNA molecules were shown to exist in agreement with the transesterification model for editing. The G6 polypeptide has limited but significant homology with a hydrophobic region of S12 ribosomal proteins. It is likely that regions G1-G5 also are pan-edited cryptogenes, indicating that pan-editing is not limited to the African trypanosome, but occurs throughout the kinetoplastidae.

C 223 THE ROLE OF INTERGENIC SEQUENCES IN THE RESOLUTION AND REGULATION OF TRYPANOSOME POLYCISTRONIC TRANSCRIPTION UNITS. Keith R.Matthews, Christian Tschudi and Elisabetta Ullu. MacArthur Center for Molecular Parasitology, Depts. of Internal Medicine and Cell Biology, Yale University, New Haven,CT. 06510.

The genomic organisation of African trypanosomes is unusual in that their genes are clustered into long polygenic transcription units driven by a distant upstream promoter. One consequence of this organisation is that the emphasis of the control of messenger RNA production is shifted from the activity of the promoter to RNA processing. This processing entails cleavage of the long precursor RNA into individual gene transcripts by trans-splicing and polyadenylation. To understand how these processes can regulate message production we have constructed a number of synthetic polycistronic transcription units containing two assayable marker genes separated by several distinct trypanosome intergenic regions. When transfected into trypanosomes the different intergenic regions result in very different levels of marker gene expression. We have begun dissecting the signals for this differential output by examining the the 3' end processing efficiencies of deletion mutants with and without the downstream signals for trans-splicing. We have found that the extent of these deletions can have severe effects upon marker gene expression and we are investigating the basis for this in detail. Additionally, we are complementing these studies by examining the RNA processing reactions on nascent RNA derived from permeablised trypanosomes. Preliminary experiments indicate a potential for interaction between trans-splicing and polyadenylation.

C 224 REQUIREMENTS FOR PRECISE AND COMPLEX POST-TRANSCRIPTIONAL PROCESSING OF BIZARRE rRNA PIECES ENCODED BY THE 6kb MITOCHONDRIAL DNA OF MALARIA PARASITES. Michael T. McIntosh, Joanne Morrisey, Akhil B. Vaidya. Malaria Research Group, Department of Microbiology and Immunology, Hahnemann University, Philadelphia, PA 19102-1192

We have previously reported the discovery and sequence of a 6kb tandemly arrayed extrachromosomal DNA in malarial parasites. The 6kb DNA appears to be a highly compressed mitochondrial genome encoding three open reading frames corresponding to cox1, cox3, and cytochrome b. In addition to these electron transport genes, highly complex and fragmented arrays of rRNA pieces are transcribed from both strands of the 6kb DNA. Northern blot and primer extension analyses show that these rRNAs are prsesent as transcripts ranging in size from about 50nt to 200nt in length. Furthermore, the rRNA pieces are found as large ribonuclear protein complexes in vivo. The scrambled rRNA gene fragments appear to encode most of the core rRNA domains of both the large and small subunit rRNAs implicated in various biochemical activities of the ribosome. Since the fragmented genes appear to lack individual promoters, generation of the pieces will require precise and complex post-transcriptional processing, followed by trans-association to form a functional ribosome. We are currently investigating the mechanisms for such processing including the possible existance of self cleaving RNA molecules. At present there is no apparent similarity to group 1 or 2 self-splicing introns nor does sequence analysis show the possible formation of hammer head structures. The possibilities of trans-association and autocatalytic or protein-assisted post-transcriptional processing will be discussed. This rRNA gene fragment distribution represents a novel processing system and possibly unique translational machinery and merits further study.

C 225 Analysis of polymorphism in the merozoite surface antigen 1 (MSA 1) of *Plasmodium chabaudi chabaudi*. Paul McKean, Kieran O'Dea and K. Neil Brown. Division of Parasitology, National Institute for Medical Research, Mill Hill, London U.K.

Previous studies in this laboratory identified a monoclonal antibody specific for the MSA 1 of the rodent malarial parasite *P. c. chabaudi* AS which, in passive transfer assays, could partially protect recipient hosts against a challenge infection with *P. c. chabaudi* AS. This same monoclonal antibody could not protect against a challenge infection with *P.c.chabaudi* CB strain parasites, nor did the monoclonal antibody recognise other *P.c.chabaudi* strains in immunoprecipitation or immunofluorescence assays.

As a continuation of this work we have been attempting to identify the epitope recognised by the protective monoclonal antibody on the MSA 1 molecule and to identify sequence differences between the MSA 1 of *P.c. chabaudi* AS and CB strains for the region encoding this protective epitope.

Epitope mapping studies have shown that the protective epitope resides near the C terminus of the MSA 1 protein, in a region predicted by nucleotide sequence analysis, of several *P.c.chabaudi* strains, to be a region of sequence diversity.

C 226 INDUCTION OF LONG LASTING T CELL REACTIVITY IN HUMAN VOLUNTEERS VACCINATED WITH KILLED M. LEPRAE VACCINE,

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Mycobacterium leprae, an obligate intracellular parasite of cells of monocyte/macrophage lineage is the causative oragnism of leprosy. Cell mediated immunity (CMI) against M. leprae antigens correlates with resistance against the disease. The incubation period for leprosy ranges from 2 to 10 years, and therefore an effective human vaccine should induce long lasting CMI. Usually the immunity induced by killed vaccines is short lived, but the CMI induced by killed M. leprae is long lasting as demonstrated by our studies. Human volunteers were vaccinated with armadillo derived killed M. leprae in 1983. Activation of M. leprae specific T cells was demonstrated at different time points upto one year of vaccination. Now, when retested after 8 years of vaccination, M. leprae reactive T cells, as demonstrated by lymphocyte proliferation assay, were still present in these volunteers. M. leprae specific CD4+ T cell clones were established and some of the antigens recognized by these T cell clones were identified from a recombinant DNA expression library. The specific epitopes were also mapped with synthetic peptides. These epitopes were recognized by relevant T cells in the context of DR MHC molecules. Some of the peptides were recognized in the context of only one DR haplotype or its subtype, whereas others were recognized in the context of multiple DR haplotypes. These epitopes may be useful in the development of specific diagnostic reagents and subunit vaccines against leprosy.

C 227 RNA EDITING COMPLEXES: RIBONUCLEOPROTEIN PARTICLES CONTAINING GUIDE RNAs, PRE-EDITED RNAs, RNA LIGASE AND TERMINAL URIDYLYLTRANSFERASE ACTIVITIES, Victoria W. Pollard, Michael E. Harris, and Stephen L. Hajduk, Department of Biochemistry, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, AL 35294

RNA editing is a post-transcriptional process found in kinetoplastid mitochondria by which immature (pre-edited) mRNAs are converted to mature (edited) mRNAs by the addition and deletion of specific uridine residues. The coding information transferred to these RNAs is thought to be specified by small anti-sense RNAs, called guide RNAs (gRNAs). Mitochondrial extracts from Trypanosoma brucei were fractionated on 10-30% glycerol gradients. Northern blot analysis indicates that guide RNAs and pre-edited RNAs co-sediment in a complex of ~40S. The guide RNAs also exhibit a lesser peak at ~19S. Treatment of the mitochondrial extract with SDS and proteinase K abolishes this pattern, indicating that the complexes have protein components. Gradient fractions were assayed for endonuclease, terminal uridylyltransferase (TUTase), and RNA ligase activities, which have been proposed to participate in the editing process. The bulk of the endonuclease existed as free protein, indicating that it is not tightly associated with the complexes. TUTase and RNA ligase, on the other hand, peaked at ~19S and shouldered into the gradient to ~40S. Treatment of the extracts with micrococcal nuclease shifts the mobilities of these activities, indicating that they are complexed with nucleic acids. We propose that two complexes exist: Complex I, consisting of gRNA, TUTase, and RNA ligase, and Complex II, consisting of gRNA, pre-edited RNA, TUTase, RNA ligase, and possibly other proteins. Complex I may be a precursor of Complex II.

C 228 CR5 MRNA IS EXTENSIVELY EDITED IN *TRYPANOSOMA BRUCEI*.

Laurie K. Read, Kenneth D. Wilson, and Kenneth Stuart. Seattle Biomedical Research Institute, 4 Nickerson St., Seattle, WA 98109-1651.

Regions of the maxicircle genome encoding transcripts which undergo RNA editing can be predicted on the basis of G vs. C strand bias. Six small G vs. C biased regions of the *T. brucei* maxicircle have been identified and termed CR1-6. We report here the edited sequence of CR5 mRNA, which was determined from the consensus sequence of more than 70 cDNAs. CR5 RNA is extensively edited by uridine insertion and deletion, and fully edited CR5 transcripts are somewhat heterogenous at their 3' ends. Edited CR5 transcripts are more abundant in bloodstream form parasites than in procyclic forms, and the implications of this developmental regulation are discussed. We have identified a CR5 gRNA from a gRNA-mRNA chimera which was generated by anchor PCR. In addition, several gRNA-mRNA chimeras from two regions of the ATPase 6 transcripts have been characterized and shown to be heterogeneous with regard to both and U tail and gRNA length.

C 229 MULTIPLE DRUG RESISTANCE (MDR) GENES IN *TRICHOMONAS VAGINALIS*, Beatrice L. Schuck, Patricia J. Johnson, Dept. of Microbiology and Immunology, Molecular Biology Institute, UCLA, Los Angeles, CA 90024. *Trichomonas vaginalis*, a flagellated protozoan, is the etiologic agent of a common sexually transmitted disease presenting as vaginitis in women. Women who are refractory to treatment have been shown to harbor parasites which are resistant to the single drug used in the U.S. for therapy (metronidazole). One possible mechanism by which *T.vaginalis* may achieve resistance is by overexpressing a P-glycoprotein-like membrane pump capable of decreasing intracellular drug concentration via ATP-dependent efflux of the drug from the cell. This mechanism of drug resistance has been found in several tumor types and over-expression of the P-glycoprotein has been correlated with drug resistance in *Plasmodium*, *Entamoeba*, and *Leishmania*. We have cloned a *T.vaginalis* P-glycoprotein gene using PCR. Northern analysis revealed that at least 4 of 7 resistant strains overexpress this mdr-like gene (tvmdr). Our analyses show that *T.vaginalis* contains two mdr-like genes that are approximately the same size as prokaryotic homologues (e.g. hlyB)- half the size of eukaryotic homologues (e.g. *Plasmodium* mdr). Additionally, certain drug resistant *T.vaginalis* strains appear to rearrange one of the mdr genes. This rearrangement is not accompanied by *DNA* amplification. The rearranged gene appears to have a chromosomal location, rather than existing as an episome. Sequence analysis has shown tvmdr to be highly similar to other genes in the P-glycoprotein family. Studies are underway to determine the relationship between tvmdr overexpression and gene rearrangement and to investigate the possible role of tvmdr in metronidazole resistance and in multiple drug resistance.

C 230 *Abstract Withdrawn*

C 231 **DEVELOPMENT OF A VACCINE AGAINST THE CATTLE TICK BOOPHILUS MICROPLUS: A BLOOD-SUCKING ECTOPARASITE, Ross. Tellam[1], Don Smith[2], Peter Willadsen[1], David Kemp[1], Keith Rand[2], Gary Cobon[2] and Michael Richardson[2].** [1]CSIRO Div. of Tropical Animal Production, Private Bag 3, Indooroopilly, QLD, Australia and [2]Biotech Australia, 28 Barcoo St., Roseville, 2069, N.S.W., Australia.

The blood-sucking cattle tick, *Boophilus microplus* transmits a number of protozoal diseases to cattle as well as having inherent debilitating effects associated with blood loss due to large infestations. Current methods of control of the tick are heavily reliant on pesticides. However, resistant strains of the tick are becoming a problem. We are developing a vaccine which immunizes cattle against the tick. The strategy used for the identification of protective antigens from the tick involves successive protein fractionations and vaccination trials. In particular, one antigen has been isolated, cloned and sequenced. The protein has 8 EGF-like modules and a hydrophobic region near the C-terminus. The latter region is involved in the addition of a glycosyl phosphatidyl inositol group which is responsible for the anchorage of this protein to the extracellular surface of the tick digest cells. Recombinant forms of this protein have been expressed in *E. coli* and insect cells and shown to be protective in vaccination trials. The locations of some of the protective epitopes in this protein have been defined by the expression of a series of recombinant proteins truncated from the C-terminus. The type of immunity developed in response to the vaccination is an example of vaccination with 'concealed' antigens.

C 232 REGULATORY ELEMENTS FOR TRANSCRIPTION OF THE U2 AND U6 snRNA GENES IN TRYPANOSOMES, Christian Tschudi[1], Amos O.Dare[1] and Elisabetta Ullu[1,2], Yale MacArthur Center for Molecular Parasitology, [1]Department of Internal Medicine and [2]Cell Biology, Yale School of Medicine, New Haven, CT 06510

The U2 and U6 small nuclear RNAs, involved in trans-splicing of pre-mRNAs in Trypanosoma brucei, are encoded by single copy genes. Inhibition studies with α-amanitin and tagetitoxin showed that both genes are most likely transcribed by RNA polymerase III. By introducing marked U2 and U6 genes into procyclic trypanosomes by electroporation, we have investigated the location of cis-acting regulatory elements. This showed that the U2 intragenic sequences are dispensable for efficient and accurate transcription. Analysis of 5' deletion mutants of the U2 gene revealed that constructs containing 174 or more base pairs upstream were transcribed, but a U2 gene with 5' flanking sequences up to -112 was inactive. We have previously shown that the -112 to -174 region contains a sequence element that is conserved both in sequence and position in the U2 genes of related Kinetoplastida. In addition, this conserved sequence element was shown to specifically bind protein factor(s). Preliminary experiments indicate that this element is orientation-dependent and that point mutations in this element reduce transcription efficiency. The 5' flanking region of the T.brucei U6 gene contains the same element. However, the region from the U6 gene cannot substitute for the U2 element. Experiments are in progress to investigate the existence of additional regulatory elements between -112 and the start site of transcription, and to characterize the trans-acting factor(s) involved in U2 transcription.

C 233 **THE EFFECT OF FLY-TRANSMISSION ON THE RATE OF ANTIGENIC VARIATION IN *TRYPANOSOMA BRUCEI*,** C. Michael R. Turner, Laboratory for Biochemical Parasitology, Department of Zoology, University of Glasgow, Glasgow G12 8QQ, UK.

The frequency with which *Trypanosoma brucei* parasites undergo antigenic variation is controversial. Low *per capita* switching rates (approximately 10^{-6}) have been observed in extensively syringe-passaged trypanosomes which contrast to the much higher rates ($>10^{-3}$) observed in cyclically-transmitted infections. These differences could be (1) intrinsic to the trypanosome genotype, (2) due to methodological differences between studies or (3) an artefact resultant from laboratory adaptation of parasites. To determine whether this laboratory adaptation of trypanosomes forms the basis for the variation in switching rates an assay has been developed, modified from a classical method for the determination of bacterial mutation rates. This assay permits determination of the rate of switching *in vivo* over a wide range of frequencies; $7 \times 10^{-7} - 4 \times 10^{-2}$ switches $cell^{-1}$ $generation^{-1}$. Using this assay, the rate of switching has been measured in a laboratory adapted cloned line of trypanosomes and compared with the rate in the same line after fly-transmission. The results showed that before fly-transmission the *per capita* rate of switching was approximately 2×10^{-6} whereas after fly-transmission the *per capita* rate was greater than 4×10^{-2}. Extensive syringe-passaging of trypanosomes appears, therefore, to lower the rate of antigenic variation by some four orders of magnitude. In fly-transmitted infections antigenic variation occurs at a very high rate; approximately 1 in 25 cells switches VAT every generation.

C 234 "EXPRESSION OF EIMERIA ACERVULINA ANTIGEN IN E. COLI, SALMONELLA AND FOWLPOX AND THE EFFECT OF IMMUNIZATION IN CHICKENS, Paul van den Boogaart, Arno N. Vermeulen~, *Jos Panhuyzen~, Alida Groenink~, Han J. Kok,* and Fiona M. Tomley*, Organon Int. BV Dept. of Biochemistry and Biotechnology, P.O. Box 20, 5340 BH Oss, The Netherlands and ~Intervet Int. BV, Dept. of Parasitology, P.O. Box 5830 AA Boxmeer, The Netherlands. * AFRC Institute for Animal Health, Houghton Laboratory, Houghton, Huntingdon, PE17 2DA, United Kingdom.

The Eimeria acervulina derived antigen Eal A (1269 bp ORF, partial gene) was expressed in different vectors. E. coli MC1061 was transformed with plasmid pMLB1113(his) in order to produce a fusion-polypeptide with a 23 amino-acid N-terminal leader which contained six histidine residues to facilitate subsequent purification by chelate affinity chromatography as described initially by Hochuli et al. (1988) Biotechnology 6, 1321-1325. Using Salmonella gallinarum 9R as a host different E. coli derived plasmids were tested. The pMLB vector described above demonstrated good expression in Salmonella, although quantitatively less than in E.coli.
For expression in Fowlpox the Eal A gene was fused to the N-terminal leader of the Newcastle Disease HN protein. The fusion protein was synthesised as an 81 kD unit. Immunization of chickens with either the subunit or the live vectors resulted in a moderate protection of 30 to 70 % reduction in oocyst output. This antigen has a very homologous counterpart in E. tenella and possibly in other species as well, being present on the refractile body membrane. Crossprotection was indeed found against lesion scores caused by E.tenella. Other species will be tested after the full length gene has been cloned.

C 235 TRANSCRIPTIONAL LINKAGE OF THE GENES FOR CALMODULIN, EF-HAND 5 PROTEIN AND UBIQUITIN-EP52 IN TRYPANOSOMA BRUCEI, Sandy Wong, Tony H. Morales, Joseph E. Neigel and David A. Campbell, Department of Microbiology and Immunology and Molecular Biology Institute, University of California, Los Angeles, California 90024. We have identified the genes for a pair of tandem, identical ubiquitin-EP52 genes and a novel EF-hand superfamily member (EFH5) in T.brucei. These genes are located downstream from a cluster of calmodulin (A,B, and C) genes. Using a PCR assay, we demonstrate the presence of RNA moieties that span the intergenic regions of 1) the UbEP52/1 and UbEP52/2 genes, 2) the EFH5 and UbEP52/1 genes, and 3) the calmodulin C and EFH5 genes. Results from a kinetic analysis of nascent RNA synthesis by UV-inactivation provide evidence that the EFH5 and UbEP52 genes are contained in the same polycistronic transcription unit as calmodulin. Together, these data confirm transcriptional linkage of these adjacent genes and suggest that non-identical housekeeping genes are transcribed in a polycistronic unit.

Molecular Biology - II and Host-Parasite Interactions - I

C 300 ISOLATION AND CHARACTERIZATION OF A UNIQUE 15KDA TRYPANOSOME SUBPELLICULAR MICROTUBULE ASSOCIATED PROTEIN, Naomi Balaban* and Rachel Goldman, Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel. *Department of Radiation Oncology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave. New York, NY 10021.
Trypanosome subpellicular microtubules form a cage-like structure of crosslinked microtubules underneath the plasma membrane. Because mammalian microtubules do not crosslink with one another, the crosslinked structure of the subpellicular microtubules is probably due to unique trypanosome microtubule associated proteins (MAPs). We have purified a 15kDa protein (p15) from *Trypanosoma brucei* subpellicular microtubules by tubulin affinity chromatography. p15 binds tubulin in tubulin overlay experiments, p15 promoted the *in vitro* polymerization (and bundling) of purified calf brain tubulin, and immunolabeling localizes p15 along microtubule polymers comprised of p15 and tubulin, as well as on the subpellicular microtubules of cryosectioned trypanosomes. Antibodies directed against p15 do not crossreact with mammalian microtubules, suggesting that p15 is a unique trypanosomal MAP. Thus, it appears that p15 is a component of the subpellicular microtubules and may be one of the elements that gives them their unique stability and structure. The uniqueness of p15 to the trypanosome and the importance of the integrity of the subpellicular microtubule to the parasite, make this protein a good candidate for therapeutic attack.

C 301 CHARACTERIZATION OF THE GENE FOR TRIOSEPHOSATE ISOMERASE OF P.falciparum, Hemalatha Balaram, Jamuna Ranie, Vidya P. Kumar, Astra Research Centre India, P.O. Box 359, Malleswaram, Bangalore 560 003, India.

In intra-erythrocytic stages of Plasmodium falciparum glycolysis to lactate is a major energy producing pathway since the single mitochondrion does not have a functional Kreb's cycle. We have cloned and obtained the full length cDNA sequence of the glycolytic enzyme, triosephosphate isomerase (TIM) from P.falciparum. Complete cDNA and genomic clones have been obtained by PCR, using a series of degenerate oligonucleotide primers coding for highly conserved polypeptide stretches in TIMs from various species. A single intron of ~ 300bp is present in the genomic clone.

The P.falciparum TIM exhibits ~ 40% homology with the human enzyme. The catalytic residues Lys(12), His(95) and Glu(165) are conserved. The polypeptide stretch from amino acids 95-105 containing the active site histidine shows significant variation from the human enzyme, an obvious difference being replacement of a totally conserved serine by phenylalanine (96) in the P.falciparum enzyme. Variations in amino acid sequence at the dimer interphase between host and parasite TIMs are also observed. This variation could be exploited to design parasite specific molecules to disrupt TIM assembly leading to inactivation of enzyme. The strategy for cloning the P.falciparum cDNA and genomic TIM sequences, significant features of the amino acid sequence and comparison with sequences from other species will be presented.

C 302 **mRNA CAP STRUCTURES OF TRYPANOSOMATIDS,** **James D. Bangs, Pamela F. Crain*, James A. McCloskey* and John C. Boothroyd, Departments of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA and *Medicinal Chemistry, University of Utah, Salt Lake City, UT.** The 5' end of mature mRNA in trypanosomatids bears, as part of a conserved sequence (the mini-exon), an extensive cap structure (CAP-4) in which the first 4 nucleotides following the triphosphate-linked m^7G are modified. Only a partial CAP-4 structure is known but its sequence and apparently its modifications are conserved among related species. We have compared [^{32}P]CAP-4 from Trypanosoma brucei and Crithidia fasciculata, purified mass amounts of Crithidia CAP-4 and have identified the component nucleosides by mass spectroscopy.

Poly A+ mRNA was decapped chemically and specifically labeled by recapping with alpha[^{32}P]GTP and guanylyl-transferase. The label can be removed by pyrophosphatase (PPase) or by RNase H/anti-sense mini-exon treatment indicating specific incorporation at the 5' end of mRNA. Following digestion with RNases (A,T_1,T_2), a labeled RNase-resistant species was isolated by DEAE chromotography. This species is CAP-4 by the following criteria: 1) [^{32}P]GMP is released by PPase treatment 2) it has the expected charge of -9 relative to standards on a MONO Q column 3) it can be generated by a procedure that involves release of label from RNA with RNase H/anti-sense mini-exon treatment. Radiolabeled CAP-4 from Crithidia and T. brucei are identical in PPase sensitivity, charge, electrophoretic mobility (25% sequencing gels) and retention in C4 reverse phase HPLC.

Labeled RNA was mixed as a marker with 10 mg of Crithidia poly A+ RNA, digested with RNases and mass amounts of CAP-4 were purified by DEAE chromatography and C4 RP-HPLC. Purified CAP-4 was converted to nucleosides by sequential digestion with nuclease P1, nucleotide PPase, and alkaline phosphatase and analyzed by combined LC-thermospray mass spectroscopy. Samples prepared by omitting PPase digestion were also analyzed. Four previously deduced (Perry et al, 1987; Sutton and Boothroyd, 1988; Freistadt et al, 1988) nucleosides (m^7G; 2'-O-mA; 2'-O-mC, A) and two novel nucleosides (m_2,2'-O-mA; m,2'-O-mU) were identified. Together with the known 5' sequence these data indicate the cap structure to be **m^7GpppAm_2AmpAmpCmpmUmpAp**. Model compounds are being synthesized to allow assignment of the base methylations in the novel nucleosides and CAP-4 from T. brucei is also being analyzed.

C 303 STAGE-SPECIFIC EXPRESSION OF NUCLEASES IN LEISHMANIA MEXICANA. Paul A. Bates, Laboratory for Biochemical Parasitology, Department of Zoology, University of Glasgow, Glasgow G12 8QQ, Scotland UK.

Promastigotes and amastigotes of Leishmania mexicana were analysed for the presence of 3'-nucleotidase/nuclease using substrate SDS-PAGE. Two forms were observed: a 40kDa band and a 29/31kDa doublet. Both were capable of using 3'AMP, 3'GMP, and poly(A) as substrates, but not 5'AMP or poly(G). Both were sensitive to inhibition with EDTA, $ZnCl_2$ and DTT, but unaffected by fluoride, tartrate or molybdate. These similarities indicated that the two forms were related, but the following differences were observed. The 40kDa band was confined to promastigotes and preferred 3'AMP as a substrate over poly(A). The 29/31kDa doublet was found at high levels in amastigotes, but was also present in promastigotes and preferred poly(A) as a substrate over 3'AMP. Cell fractionation into membrane and soluble components revealed the 40kDa band in the former and the 29/31kDa doublet in the latter. Comparable results were observed using Triton X-114 extraction, with the 40kDa band and 29/31kDa doublet fractionating into the detergent-rich and detergent-poor phases, respectively. The possibility that the 29/31kDa doublet represented proteolytic products of the 40kDa form was eliminated by testing a variety of proteinase inhibitors and by examining the effects of mixing of amastigote and promastigote homogenates together. Further, transformation experiments showed that the two forms were under stage-specific regulation: the morphological differentiation of promastigotes to amastigotes and vice-versa was accompanied by the loss of one form and increase of the other during growth in vitro. The 40kDa band is likely to be the surface-membrane enzyme described in promastigotes of other species with a presumed role in purine salvage. The 29/31kDa doublet has not been reported before and probably represents a soluble, lysosomal homologue. It is expressed at higher levels in amastigotes may play a role in survival of these forms within the phagolysosome.

C 304 *FASCIOLA HEPATICA*: THE EFFECTS OF INFECTION ON THE BIOENERGETICS OF LIVER CELLS IN THE RAT, Carolyn A. Behm, Linda M. Lenton and Fyfe L. Bygrave, Division of Biochemistry and Molecular Biology, School of Life Sciences, Australian National University, Canberra, Australia 2601

Mitochondria isolated at 4 weeks post-infection from the livers of rats infected with *F. hepatica* do not synthesise ATP *in vitro*, as shown by ^{32}P incorporation experiments with succinate as oxidisable substrate. This observation correlates with the observations from our group (Rule *et al.* 1989 *Biochem. J.* 260, 517 and manuscript in preparation) that respiration in isolated mitochondria and in isolated hepatocytes is uncoupled at this stage of the infection, and confirms that bioenergetic metabolism in cells from infected livers is compromised. Treatment of infected rats with dexamethasone restores the rate of *in vitro* mitochondrial ATP synthesis to normal levels, indicating a possible role of the host's cellular inflammatory response in the development of the bioenergetic lesions. The F_1F_O ATP synthetase complex in the mitochondria from infected livers is abnormal. Although the total activity of the synthetase, assayed as ATPase activity, in isolated liver mitochondria does not change, the enzyme complex loses sensitivity to the proton transport inhibitors oligomycin, DCCD and diethylstilbestrol. The loss of sensitivity to oligomycin and DCCD is maximal at at 3-4 weeks post-infection and diminishes thereafter to reach normal levels at 10 weeks post-infection. Loss of sensitivity to these inhibitors is evidence for a possible structural alteration in the complex or one of its subunits. Analysis of the subunit composition of the F_1F_O ATP synthetase from submitochondrial vesicles or from purified preparations of the complex is in progress.

C 305 AFFINITY PURIFICATION AND DOMAIN ANALYSIS OF THE *TRYPANOSOMA BRUCEI* SMALL NUCLEAR RIBONUCLEOPROTEINS (snRNPS) Albrecht Bindereif, Mike Cross, Arthur Günzl, Andreas Missel, and Zsofia Palfi, Max-Planck-Institut für Molekulare Genetik, Otto-Warburg-Laboratorium, D-1000 Berlin 33.

We have purified each of the *trans*-spliceosomal snRNPs (SL, U2, U4/U6) from *T. brucei* extracts, using affinity selection with biotinylated anti-sense 2'-OMe RNA oligonucleotides. A set of five common and several snRNP-specific protein components were identified. Polyclonal antibodies were raised against each of two U2 snRNP-specific and against four common snRNP proteins. U2-specific antibodies recognized only the U2 snRNP, whereas the antibodies against the common snRNP proteins immunoprecipitated SL, U2, U4, U6, and several additional RNAs. No significant immunological relationship between the *T. brucei* U2 snRNP proteins and human snRNP proteins could be detected.

snRNP protein binding sites have been mapped to the 3' half in each of the SL, U2, and U4 snRNAs by a combination of RNase H protection and Northern analysis. By *in vitro* reconstitution and chemical modification-interference assays discrete positions within stem-loop IV of U2 RNA were identified that are essential for protein binding; these data suggest that there are *trans*-spliceosomal specific RNA-protein interactions. In addition, based on immunoprecipitation/RNase T1 protection assays, the adjacent single-stranded region appears to be involved in protein binding.

Through the use of specific antibodies and peptide sequences we are currently isolating and characterizing the *T. brucei* genes for U2 snRNP proteins, which should enable a more detailed comparison between *cis*- and *trans*-spliceosomal snRNPs.

C 306 POTENTIAL HEXOSE TRANSPORTER GENES EXPRESSED PREDOMINATELY IN THE BLOODSTREAM FORM OF TRYPANOSOMA BRUCEI, Frederic Bringaud and Theo Baltz, Laboratoire d'Immunologie et de Parasitologie Moleculaire, Universite de Bordeaux II.
A cDNA cloned from Trypanosoma brucei brucei codes for a putative membrane protein which is homologous to the erythrocyte glucose transporter and several other sugar transporters from Escherichia coli, yeast, algae and Leishmania. This cDNA hybrizes to a 2,3 kb mRNA that accumulates to a much higher degree in the bloodstream mammalian form than in the procyclic insect form of the parasite. The correlation between the expression of this gene and the hexose metabolism of Leshmania enrietti and T. brucei suggest that these two related genes probably encode hexose transporters. The gene encoding this mRNA is a member of multigene family. The putative hexose transporter gene is highly conserved among Kinetoplastidae, indicating an important role for this protein in the parasite life cycle.

C 307 SECRETED AND SOMATIC PROTEINASES OF THE BOVINE LUNGWORM DICTYOCAULUS VIVIPARUS AND THEIR INHIBITION BY ANTIBODY FROM INFECTED AND VACCINATED CATTLE. Collette Britton[1], David P. Knox[2], George M. Urquhart[3] and Malcolm W. Kennedy[1]. [1]Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden Road, Glasgow, G61 1QH., Scotland, U.K., [2]Moredun Research Institute, Edinburgh, Scotland, and [3]Department of Veterinary Parasitology, University of Glasgow, Scotland, U.K.

Stage-specific proteinase activities have been detected in extracts and excretory-secretory (ES) products of the infective and adult stages of the cattle lungworm, Dictyocaulus viviparus. Multiple enzyme activities have been identified from each source, as defined by pH optima, substrate specificities, inhibitor effects and substrate gel electrophoresis. The antigenicity of these parasite proteinases was demonstrated by the inhibition of their enzymic activity by Protein G-purified serum antibody from infected and vaccinated hosts. The antibody response to these proteinases might be involved in protective immunity or limitation of tissue damage by materials released from the parasite. Work is currently underway to clone gene fragments encoding cysteinyl proteinases of D. viviparus by polymerase chain reaction (PCR) amplification using oligonucleotides complimentary to the conserved amino acid sequences around the catalytic site.

C 308 MOLECULAR CLONING AND CHARACTERIZATION OF A 42 KDA PROTEIN PHOSPHATASE OF LEISHMANIA CHAGASI. James M. Burns, Jr., Marilyn Parsons, Donna M. Russo, Diane E. Rosman, and Steven G. Reed. Seattle Biomedical Research Institute, Seattle, WA 98109 and Cornell University Medical College, New York, NY 10021.

This laboratory previously identified a 42 kDa glycoprotein of Leishmania chagasi which consistently elicited strong proliferative responses as well as IL-2 and IFN-γ production from T lymphocytes from leishmaniasis and Chagas' disease patients. A rabbit serum raised against purified Lc gp42 was used to screen a L. chagasi genomic expression library. One of the isolated clones produced a 38 kDa fusion protein which was was purified by preparative isoelectric focusing and ammonium sulfate precipitation. The purified protein elicited strong in vitro proliferative responses from PBMC of patients with histories of leishman-iasis but not from normal PBMC. Immunoblot analysis using a polyclonal rabbit serum raised against the recombinant antigen identified the native molecule as a 42 kDa protein present in L. chagasi and L. amazonensis promastigotes and amastigotes as well as T. cruzi epimastigotes. By Southern analysis, homologous sequences were detected in the genomes of L. chagasi, L. amazonensis, L. braziliensis, L. donovani, and L. major. DNA sequence analysis of a clone containing the complete gene sequence revealed an open reading frame encoding 399 amino acids with significant homology to a 42 kDa rat type 2C protein phosphatase. Further characterization of human T cell responses to a full length recombinant gene product as well as the analysis of enzymatic activity of the recombinant protein are in progress. This work was supported by NIH grant AI25038.

C 309 ISOLATION OF A cDNA FOR NUCLEOSIDE-PHOSPHATE KINASE FROM SCHISTOSOMA MANSONI, Ming Cao, Robert Akridge, Dave Weston, Walter M. Kemp and Barbara L. Doughty, Department of Veterinary Pathobiology, College of Veterinary Medicine and Department of Biology, College of Science, Texas A&M University, College Station, TX 77843
Nucleoside-phosphate kinases catalyze the reversible phosphorylation of nucleotides according to the equation NTP+NMP=NDP+NDP. They are involved in a variety of functions such as maintenance of metabolic energy sources and biosynthesis of ribonucleotides. A cDNA encoding for part of the nucleoside phosphate kinase was cloned from a S. mansoni egg cDNA expression library constructed in lambda ZAP II vector by using a polyclonal antiserum specific for S. mansoni egg antigens. The restriction enzyme EcoR1 and EcoRV fragment from the insert of this clone was radiolabeled and used as a probe to rescreen the egg and adult worm cDNA libraries. This procedure yielded 12 positive clones with sequences extending from both the 5' and 3' end of the original clone. The longest sequence generated from these clones has 1238 base pairs(bp) containing a 582 bp coding region, a 89 bp 5' and a 558 bp 3' non-coding regions with a poly A tract. The deduced peptide has a molecular weight of 22 kilodaltons. It shows a higher degree of homology (67-69%) with mammalian adenylate kinase 1(AK1) than with AK2 and UMP-CMP kinases(51-61%). It also lacks the insertion of about 30 amino acids that is found in the middle of the AK2 and AK3. This fact suggests that this gene encodes a AK1 from S. mansoni.

C 310 MOLECULAR STUDIES ON THE ROLE OF ICAM-1 AS A RECEPTOR FOR PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES. Alister G. Craig, Anthony R. Berendt, *Alison McDowall, *Paul A. Bates *Nancy Hogg and Christopher I. Newbold, Molecular Parasitology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, OX3 9DU, U.K. *Macrophage Laboratory,ICRF Laboratories, P.O. Box123, London, WC2A 3PX, U.K.

The intercellular adhesion molecule-1 (ICAM-1) is one of three putative endothelial receptors which mediate *in vitro* cytoadherence of *Plasmodium falciparum* -infected erythrocytes. As cytoadherence to post-capillary venular endothelium is thought to play a major part in the virulence of *P. falciparum* malaria, we have examined the interaction between ICAM-1 and the infected erythrocyte. Three anti-ICAM-1 mAbs which block *in vitro* binding of infected erythrocytes to ICAM-1 have no effect on the interaction between LFA-1 and ICAM-1 suggesting that the binding sites on ICAM-1 for these two ligands are distinct. Mapping of one of these blocking mAbs to an octapeptide together with expression of domain-deletion constructs and homologue-scanning mutagenesis have shown that a region of domain 1 of the ICAM-1 molecule is a critical part of the *P. falciparum* -infected erythrocyte binding site. Modelling the two N-terminal domains (based on an alignment of the ICAM-1 sequence with that of the REI kappa light chain and the second domain of CD4) has suggested that this region is on the side of domain 1 opposite to that involved in the interaction with LFA-1. Furthermore, we have made a soluble ICAM-1-Fc chimera for further studies on the interaction *in-vitro* with laboratory-adapted and field isolates. These findings have implications in relation to the nature of the interaction between the infected erythrocyte and post-capillary venular endothelium and for the development of therapeutic reagents which may block the adhesion of *P. falciparum* -infected erythrocytes but not affect LFA-1/ICAM-1 mediated immune functions.

C 311 THE HUMAN HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE (HPRT) AND THE TARGETED INHIBITION OF PARASITIC ENZYMES Sydney P. Craig III[1], Ling Yuan[1], Christopher Bystroff[2], Pamela Focia[1], Robert Fletterick[2] and Ching C. Wang[1], Departments of Pharmaceutical Chem.[1] and Biochem. & Biophysics[2], UCSF, San Francisco, CA 94143

Because the majority of parasites examined lack *de novo* pathways for the synthesis of purine nucleotides, these organisms are forced to rely exclusively upon the salvage of exogenous purine bases or nucleosides to supply purine nucleotides essential for cellular metabolism. The enzymes responsible for purine salvage have been proposed as targets for the chemotherapeutic treatment of a number of parasitic diseases including malaria, leishmaniasis, giardiasis, Chagas' disease, and schistosomiasis [see Craig *et al.* (1991) in *Molec. & Immunol. Aspects of Parasitism*, ed. by C.C. Wang, A.A.A.S., Washington, D. C., 123-138]. For this reason, DNA's encoding the purine salvage enzymes of several different parasites have been cloned and a few have been expressed in bacteria with the objective of discovering differences between the parasitic and human enzymes that might be exploited in the design of drugs targeted to the enzymes of the parasites. With this goal in mind, as it applies to schistosomiasis, we have cloned, expressed, purified and crystallized the schistosomal HPRT, and have measured X-ray diffraction data to 2.9 Å resolution. However, a missing element for these studies has been a comparable expression system for the human HPRT; one that could be used for drug screening as well as for the generation of large quantities of enzyme for crystallization and the determination of three dimensional structure. Thus, recombinant expression of the human enzyme has been considered essential for both the rational design and screening of drugs specifically targeted to parasitic HPRT. Herein, using the pBAce vector [Craig *et al.* (1991) *Proc. Natl. Acad. Sci.* **88**, 2500-2504]we report the successful high level expression in *Escherichia coli* of soluble, enzymically active human HPRT (>60 mg or >1,000,000 units per liter of culture). Using a combination of heat treatment and HPLC chromatography in monoQ and monoP columns from Pharmacia, the recombinant human enzyme has been purified to homogeniety, and crystals have already been generated in preparation for X-ray crystallographic analysis. In addition to facilitating structural studies for the human HPRT, the continued availability of an abundant source of the human enzyme will enable extensive steady state kinetic studies and the rapid screening of substrate analogs as prospective drugs for the treatment of parasitic disease.

C 312 IDENTIFICATION OF A GLUTAMATE AND AVERMECTIN-SENSITIVE CHLORIDE CHANNEL EXPRESSED IN *XENOPUS* OOCYTES INJECTED WITH mRNA FROM THE NEMATODE *CAENORHABDITIS ELEGANS*, Doris F. Cully, Philip S. Paress, Ken K.Liu, and Joseph P. Arena, Biochemical Parasitology, Merck & Co., Rahway, NJ 07065

The avermectins (AVM) are a family of macrocyclic lactones with anthelmintic and insecticidal activity. To investigate the site and mechanism of action of these compounds we have used as a model the free living nematode *Caenorhabditis elegans*. *C. elegans* is highly sensitive to the AVMs and contains a membrane associated high affinity AVM binding site (K_D=0.2 nM). We have used the *Xenopus* oocyte for expression of *C. elegans* mRNA and have identified a glutamate and AVM-sensitive membrane current. The current was unchanged in oocytes injected with EGTA and exhibited a reversal potential of -19 mV that shifted in low chloride solutions as predicted for a chloride channel. When maximal concentrations of AVM and glutamate were used the response was less than additive, suggesting they both activate the same channel. Both the glutamate and AVM-sensitive channels were blocked by picrotoxin but insensitive to the neurotransmitter GABA. Size fractionation of *C. elegans* mRNA on agarose gels showed that RNA encoding the AVM and glutamate-sensitive channel was found in the 1.8-2.0 Kb mRNA class. A cDNA library will be synthesized and used to screen for depletion of AVM-sensitive current in oocytes.

C 313 ANALYSIS OF A FAMILY OF RIBOSOMAL GENES IN *BABESIA BOVIS*; USE OF A RECOMBINANT GST-L12eI ACIDIC RIBOSOMAL PROTEIN IN A CATTLE VACCINATION TRIAL, Brian P. Dalrymple, Donald A. Berrie, Jenny M. Peters, Christine Dimmock, David J. Waltisbuhl, Graham Leatch, Kurts Rode-Bramanis and Ian G. Wright. CSIRO Division of Tropical Animal Production, PO Box #3, Indooroopilly, Queensland, 4068, Australia.

A cDNA clone encoding a *Babesia bovis* ribosomal protein was isolated from a λgt11 expression library. The 112 amino acid protein has all the features of the L12eI group of acidic ribosomal proteins. A recombinant glutathione-S-transferase (GST-L12eI) fusion protein of the complete amino acid sequence has been expressed and purified. Using rabbit antiserum raised against this protein the native *B. bovis* protein of apparent mw 17,000 has been identified. The gene encoding this protein has been designated L12eIA. A closely related gene, probably encoding a second L12eI protein homologue (L12eIB), and two more distantly related genes have also been identified. One of the latter genes appears to correspond to a previously described *B. bovis* recombinant cDNA clone, K_ABb1. This gene has now been identified as encoding a putative L10e ribosomal protein homologue. The fourth gene identified probably encodes an L12eII acidic ribosomal protein homologue. Analysis of genomic DNA and clones of genomic DNA suggests that the members of this family of genes are not directly adjacent in the genome.

An ELISA assay using the GST-L12eI fusion protein has been developed. Forty steers previously unexposed to *B. bovis* were assayed before and after infection with *B. bovis*. More than half of the animals had significant antibody binding to the recombinant fusion protein after infection. Five steers previously unexposed to *B. bovis* were vaccinated with the recombinant fusion protein. The animals were challenged with a heterologous virulent line of *B. bovis* parasites. No significant difference in PCV falls and temperature rises were observed between the controls and the vaccinated animals. However, a significant reduction in parasitaemias in the vaccinated group was observed.

C 314 THE CHARACTERIZATION OF THE SMALL HEAT SHOCK COGNATES IN THE MICROFILARIAE OF *BRUGIA PAHANGI*. E. Devaney, R. Jecock, E. Lewis and A. Egan. Department of Parasitology, School of Tropical Medicine, Liverpool, L3 5QA.

The first stage larvae or microfilariae (mf) of the filarial nematode *B. pahangi* are a developmentally arrested life cycle stage, circulating in the mammalian host. The developmental cycle is re-initiated only after ingestion by an appropriate mosquito vector. Comparison of the profile of proteins synthesized in mf cultured under mammalian (37°C) and mosquito (28°C) conditions demonstrated that mf synthesize a set of polypeptides homologous to the small heat shock proteins (hsps) described from other other organisms only at 37°C. These molecules are synthesized in mf cultured under non-stress conditions and are therefore referred to as heat shock cognates (hscs). They are a major protein product of the mf at 37°C and are immediately repressed upon transfer of the mf to mosquito culture conditions (28°C). Their synthesis appears to be under strict developmental control as they are not observed in the mature adult female, where much of the protein synthesis is destined for the mf developing *in utero*. A possible link between the expression of the small hscs and the re-initiation of mf development is suggested by the observation that in infected mosquitoes held at 37°C, development of the mf beyond the late first larval stage is inhibited.

C 315 IDENTIFICATION AND MOLECULAR CLONING OF THE GENE FOR THE MAJOR *CRYPTOSPORIDIUM PARVUM* ANTIGEN RECOGNIZED BY HYPERIMMUNE BOVINE COLOSTRUM, Patricia S. Doyle, Jiri Gut, Richard G. Nelson, James H. Leech, Joseph H. Crabb and Carolyn Petersen. Parasitology Laboratory, San Francisco General Hospital and Departments of Medicine and Pharmaceutical Chemistry, University of California at San Francisco, San Francisco, California and ImmuCell, Portland, Maine.

Cryptosporidium parvum, a parasitic protozoan, causes self-limited diarrhea in immunocompetent persons and life-threatening dehydration and cachexia in persons with AIDS. Hyperimmune bovine colostrum (HBC) IgG raised to *Cryptosporidium* oocysts has been reported to cure some AIDS patients and protects against sporozoite challenge of calves. However, sufficient quantities of effective HBC IgG have not become commercially available for treatment of patients with cryptosporidiosis.

As a preliminary step in characterizing the protective antigens of *Cryptosporidium* recognized by HBC IgG and expressing them in sufficient quantities to serve as specific immunogens, we have identified 12 major proteins of oocysts and sporozoites on Western blot with HBC IgG. The dominant antigen is a M_r >500 kD protein. Cross immunoprecipitation/competition experiments using HBC IgG and monoclonal and polyclonal antibody to the M_r > 500 kD protein indicate that this protein is a Triton X-100 soluble glycoprotein of sporozoites and merozoites previously identified and characterized in our laboratory. N-glycosidase F treatment of the glycoprotein indicated that the protein is heavily N-glycosylated and that the M_r of the protein portion is <200 kD. The gene for the glycoprotein has been cloned and partially sequenced.

C 316 ROLE OF METACESTODE-DERIVED PROTEIN(S) IN THE HOST ANTIBODY RESPONSE, D. Mark Estes and Judy M. Teale, Department of Microbiology, University of Texas Health Science Center, San Antonio, TX 78284-7752

Mice infected with the cestode parasite *Mesocestoides corti* develop severe hypergammaglobulinemia. Previous studies have shown that the antibody response generated as a result of infection is restricted to IgM and IgG1 isotypes and is T cell dependent. To test the role of the parasite antigens themselves in the isotype restricted response, mice were immunized with intact but nonviable organisms. In this case, it was found that multiple isotypes were produced including IgG2a indicating that the viable organism and an active infection were critical to the isotype restricted response. These findings and others suggested that the parasite sheds or secretes molecules that are essential to the selective induction of IgM and IgG1 antibodies. Two proteins isolated from *M. corti* culture supernatants were found to be homologous to the 70kD heat shock proteins (hsp70) and E. coli GroEL families of stress proteins. Lymphocytes from infected mice proliferate in response to complete supernatatant and both the isolated p60 and p70 stress protein homologues. In addition, stress protein containing supernatant from *M. corti* cultures stimulates an in vitro antibody response restricted to IgM and IgG1; the same isotypes induced during infection. These results suggest that stress proteins play an integral part in the immune response to *M. corti* and the associated isotype restriction.

C 317 **IDENTIFICATION OF A CUTICULAR SURFACE ANTIGEN CONSERVED IN A RANGE OF NEMATODES OF VETERINARY IMPORTANCE,** **John S.Gilleard, Andrew Tait and James L.Duncan.** Department of Veterinary Parasitology, Glasgow University, Glasgow, Scotland, U.K.

The bovine lungworm Dictyocaulus viviparus induces a highly effective immune response and an irradiated larval vaccine has been in use for 30 years. Studies have been initiated to identify the antigens involved in immunity. These have shown that sera from immune cattle have a surprisingly high titre of antibody to the retained L2 cuticle surface but only a low titre to the infective L3 cuticle surface. In order to define and identify the antigens concerned six monoclonal antibodies were generated against the retained L2 cuticle surface; all recognise a smear between 29-45kDa on Western blots. Immunoblotting and immuno-precipitation studies with immune bovine sera show this to be the most immunodominant soluble antigen in the L3. The exposure of the antigen on the cuticle surface is entirely stage specific whereas its presence internally is not. Immunofluorescence studies show the monoclonal antibodies bind to the surface of the retained L2 cuticle (which covers the infective L3) in all nematodes examined which possess such a structure. These include ruminant nematodes such as Haemonchus contortus, Trichostrongylus colubri-formis, Ostertagia circumcincta, Ostertagia ostertagi, Cooperia oncophora, Nematodirus battus and Cyathastomum species of horses. The number and molecular weights of the polypeptides on Western blots varies between the different species. These results indicate that we have identified a cuticular surface antigen which is immunodominant in Dictyocaulus viviparus infection and is conserved in a stage and site specific manner in numerous different nematode species.

C 318 *Cryptosporidium parvum:* IDENTIFICATION OF INTRACELLULAR TROPHOZOITE AND MERONT ANTIGENS WITH MONOCLONAL ANTIBODIES. Jiri Gut, Patricia S. Doyle, Carolyn Petersen, Richard G. Nelson, and James H. Leech. Parasitology Laboratory, Departments of Medicine and Pharmaceutical Chemistry, San Francisco General Hospital and the University of California, San Francisco, CA.

C. parvum is an intracellular coccidian parasite that infects the microvillous border of intestinal epithelial cells and causes diarrhea. Most previous studies of *C. parvum* biology have focused on the readily accessible extracellular stages (oocysts and sporozoites). However, intracellular stages (trophozoites and meronts) may have a greater role in the pathogenesis of disease and may be important targets of host immune responses.

As a first step in the investigation of intracellular parasite biology and immunology, we report the production of monoclonal antibodies (Mabs) to trophozoite and meront antigens of *C. parvum*. We used *C. parvum* cultured in Madin-Darby canine kidney (MDCK) cells as the immunogen and screened the hybridomas by immunofluorescence with fixed, infected MDCK cells. Seven Mabs that react specifically with structures of intracellular trophozoites and/or meronts have been isolated. Five Mabs recognize meront antigens that cross react with sporozoite/oocyst antigens as determined by their reactivity with oocyst/sporozoite Western blot proteins of Mr >500 kD (MAbs M2, M15), 300 kD (MAb M6), 50 kD (MAb 20) and 14/35 kD (MAb 9). Two MAbs (MAbs M8 and M24) do not react with oocyst and sporozoite proteins and therefore appear to be specific for intracellular stages.

In previous work we have developed 3 MAbs, using oocysts/sporozoites as immunogens, to a Mr >500 kD sporozoite glycoprotein. The MAbs to the >500 kD sporozoite glycoprotein reacted on fixed IFA with intracellular parasites. It appears that we have developed more reagents, using intracellular parasites as immunogens, to this highly immunogenic glycoprotein which is shared by sporozoites and intracellular parasites.

C 319 LYSIS OF TRYPANOSOMA BRUCEI BY A TOXIC SUBSPECIES OF HUMAN HIGH DENSITY LIPOPROTEIN REQUIRES RECEPTOR MEDIATED ENDOCYTOSIS, Kristin Hager, Mark Pierce, Ray Moore, Ewan Tytler, Jeff Esko and Steve Hajduk, Department of Biochemistry, University of Alabama at Birmingham, School of Medicine, Birmingham, AL 35294

Trypanosoma brucei brucei, the causative agent of Nagana in cattle in sub-Saharan Africa is not pathogenic to humans due to its sensitivity to lysis by human serum. This serum sensitivity may be the principle phenotypic difference between *T. b. brucei* and the human sleeping sickness parasites *T. b. rhodesiense* and *T. b. gambiense*. The lytic activity in human serum was associated with high density lipoprotein (HDL) (Rifkin, M. R., 1978). The Trypanosome Lytic Factor (TLF) is a minor, heterogeneous and very dense subclass of HDL which is characterized by the presence of two unique disulphide-linked apoproteins apo LI and LIII. TLF activity can be thermally inactivated or abolished by trypsin treatment. Detergent-mediated reconstitutions with TLF components show that LIII is the required toxin and implicate LI as the putative ligand for binding. We present evidence that lysis of *T. b. brucei* occurs via receptor-mediated endocytosis and present a model for the lytic pathway. Lysis is temperature dependent and there is a lag period before the onset of lysis. ^{125} I-TLF binds to *T. b. brucei* in a saturable and biphasic manner. Binding is competed by excess cold TLF and by non-lytic HDL. Au-conjugated TLF concentrates in the flagellar pocket and is subsequently endocytosed in vesicles in close proximity to the flagellar pocket. We propose that cell lysis requires the uptake and fusion of endocytic vesicles with lysosomes. Treatment of the cells with lysosomotropic amines prior to incubation with TLF completely protects the cells from lysis as does incubation of the cells with TLF at 17^{0}C. The endocytic pathway will be biochemically defined by cell fractionation to better understand the mechanism of TLF lysis.

C 320 BIOCHEMICAL ASPECTS OF THE INHIBTION OF POLYCLONAL MOUSE B CELL ACTIVATION BY PHOSPHORYLCHOLINE (PC)- CONTAINING FILARIAL EXCRETORY-SECRETORY ANTIGENS, W. Harnett, Department of Immunology, University of Strathclyde

E-S 62, the major excretory-secretory product of the rodent filarial parasite, *Acanthocheilonema viteae*, is a member of the PC-containing family of filarial E-S. It is particularly suitable for analysis of the properties of these molecules, as it can be readily obtained in relatively large amounts in purified form. In view of the current suspicion that PC may act as an immunosuppressive agent in filariasis, E-S 62 has been investigated for immunomodulatory activity. It was observed that E-S 62 was able to inhibit activation of small resting murine B cells by $F(ab)_2$ fragments of rabbit antibodies directed against the Fab region of mouse IgG. Such fragments interact with the Fab region of immunoglobulin (Ig) on the B cell surface, and hence can be considered as a model form of antigen. Experiments in which E-S 62 was substituted by PC bound to BSA (PC-BSA) or PC alone demonstrated that these molecules also possessed inhibitory effects. This indicates that it is likely to be the PC group of E-S 62 which is responsible for inhibition.

Murine B cells can also be activated polyclonally by lipopolysaccharide, but PC is unable to interfere in this system. LPS does not activate cells via surface Ig receptors, which suggests that these may be important in relation to the inhibitory effect. One of the earliest biochemical events following ligation and cross-linking of surface Ig receptors on B cells is the hydrolysis of phosphatidylinositol bisphosphate to generate the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 mobilises intracellular stores of calcium and DAG is an activator or Protein Kinase C (PKC). It has been found that PC does not inhibit the generation of IP_3 associated with ligation of the surface Ig receptors. This result suggests that PC is likely to be acting downstream of these early events.

The effect of PC may in fact focus on PKC. PKC activity within B cells is increased, following engagement of surface Ig receptors but, interestingly, this appears to be much reduced in the presence of PC. It has also been observed that resting cells cultured with PC alone demonstrate reduced PKC activity. PC-mediated downregulation of PKC levels will probably result in the inhibition of further events mediated by this important regulatory enzyme, and this may explain the inhibition of B cell proliferation induced by PC. The mechanism by which PC downregulates PKC levels remains to be established.

C 321 DEMONSTRATION OF A CAPPING-LIKE PHENOMENON ASSOCIATED WITH THE TEGUMENTAL SURFACES OF ADULT MALE *SCHISTOSOMA MANSONI*, W. M. Kemp*, R. E. Akridge*, A. Goes^, and B. L. Doughty^, Departments of Biology* and Veterinary Pathobiology^, Texas A&M University, College Station, Texas 77843.

A time and temperature dependent lateral movement of immunocompromised tegumental surface associated antigens was documented and partially characterized by immunocytochemical techniques. Compromised antigens were translocated in a highly organized manner to the apex of the dorsal tubercles on adult male *S. mansoni* immediately prior to the expulsion of these antigens from the parasite. This translocation process was blocked by vinblastin, cytochalasin B, calmodulin inhibitors (R24571 and trifluoroperazine) and the ionophore, monensin. Praziquantel accelerated the capping process, but inhibited the shedding of the capped antigens. The binding of a human monoclonal antibody (B10), which is specific for a 28 kDA adult male surface antigen, did not initiate either the translocation or the shedding mechanisms. (Supported by NIH, NIAID Grant #AI26505 and Texas Advanced Research Program Grant #010366-100)

C 322 RAPID TRANSFORMATION OF NEMATODE SURFACE LIPID AFTER EXPOSURE TO MAMMALIAN TISSUE CONDITIONS.

Malcolm W. Kennedy, Lorna Proudfoot, Huw V. Smith, William Harnett, Michael J. Worms and John R. Kusel. Wellcome Laboratories for Experimental Parasitology and Department of Biochemistry, University of Glasgow, Bearsden, Glasgow G61 1QH, Scotland, U.K.

The surface lipid of mammalian-parasitic stages of nematodes is unusual in that it is highly selective to the insertion of fluorescent lipid analogues. To date, only 5-N-(octadecanoyl)aminofluorescein (AF18) and nitrobenzoxadiazole-cholesterol (NBD-chol) have been found to insert in a surface-restricted manner. Infective larvae, however, appear to be radically different in that they label with none of the probes tested, and this applies to both free-living or vector-borne larvae (*Brugia pahangi, Acanthocheilonema viteae, Strongyloides ratti, Nippostrongyloides brasiliensis, Trichinella spiralis* and *Ostertagia ostertagi*). The change to a lipophilic surface can occur in as little as 10 min for vector-borne larvae, and in a matter of hours for the others. During this transition, lateral mobility of AF18 is detectable by Fluorescence Recovery After Photobleaching (FRAP) in infective larvae of *A. viteae*, but not in later developmental stages or microfilariae. In larvae of *S. ratti*, the acquisition of affinity for AF18 is accompanied by the rapid shedding of an anionic surface coat. The stimuli for the *in vitro* induction of the transformation include pH, temperature and sodium ion concentration in the medium. Modifiers of second messenger signalling pathways have been used to provide evidence that the Na^+/H^+ antiport system, changes in intracellular calcium, cAMP and cGMP are involved in mediating the transformation.

C 323 CHARACTERIZATION OF A TOXOPLASMA MUTANT WITH DECREASED VIRULENCE.

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A Toxoplasma surface antigen (p30 or Sag 1) mutant was generated by ethylnitrosourea mutagenesis of tachyzoites and selection with complement and a monoclonal specific for p30 (2F2). The resulting mutant, *sag1-3*, was cloned twice by limiting dilution before further analysis. This mutant expressed an altered p30 with significantly reduced mobility compared with wild-type p30 on unreduced gels probed with polyclonal rabbit p30 antisera and p30-specific monoclonals. The difference in mobility was less pronounced on reduced gels. p30 was no longer detectable on the parasite surface when IFA was performed on live parasites with polyclonal rabbit sera and monoclonal antibodies. Fixed permeabilized parasites, however, were positive by IFA with polyclonal sera and monoclonal 2F2 confirming that p30 is expressed in *sag1-3*. Approximately 30% of mutants passaged in HFF developed a grossly abnormal open rosette configuration within the parasitophorous vacuole. The mutant also grew more slowly in HFF than its parent strain when growth was measured by uracil uptake. *sag1-3* was significantly less virulent than its parent; its LD50 in inbred and outbred mice was 100-1000 fold greater than that of wild-type. Further studies are in progress to establish the role that the altered p30 expressed by *sag1-3* has upon the observed decreased growth rate and virulence of the mutant.

C 324 **A ROUGH GUIDE TO DIFFERENTIATION FROM MACROSCHIZONT TO EXTRACELLULAR MEROZOITE IN THEILERIA ANNULATA.**

Jane Kinnaird, Joanne Dickson, Susan McKellar, Lawrence Tetley, Andrew Tait and Brian R.Shiels. Wellcome Unit of Molecular Parasitology, Glasgow University, Glasgow, Scotland, U.K.

Theileria annulata is a tick transmitted parasite which infects cattle in many parts of Africa, Asia and Europe. In the intra-leukocytic stage the parasite exists as a multinucleate macroschizont which can undergo differentiation to produce numerous extra cellular merozoite particles which invade red blood cells. Differentiation to the merozoite can be induced in vitro by culture at 41°C. Our experiments are directed towards an understanding of the regulation of this process at the molecular level. From a low passage mixed stock we have derived cloned cell lines, some of which differ in their abilities to differentiate. In clones D7 and C9 >90% of the cells undergo differentiation with a high level of merozoite production after 8-10 days of culture at 41°C. Using polyspecific antisera and monoclonal antibodies, novel polypeptides are found from day 4 of culture at 41°C. A major protein of 30kd is observed early on and is expressed on the surface of the developing macroschizont. This protein is also present on the surface of the mature merozoite. Another major antigen of 110kd is expressed later in differentiation and has been found by immunoelectron microscopy to be localised in the rhoptry. Using these proteins as markers we have found that differentiation to the merozoite is a two-phase process. By inducing the culture at 41°C for defined periods of time followed by continued culture at 37°C we find that the first stage is reversible. However, after 4 days of culture at 41°C some form of 'committment' occurs and differentiation is no longer reversible.

C 325 CHARACTERISATION OF THE *SCHISTOSOMA MANSONI* CATHEPSIN B IN THE BACULOVIRUS EXPRESSION VECTOR SYSTEM,

Mo Klinkert and Bernhard Götz, Institute for Cell Biology, Consiglio Nazionale della Ricerche, Rome, Italy

In schistosomes a variety of proteinases are thought to be involved in the degradation of host haemoglobin for nutrition. The major activity has been defined as that of a cathepsin B-like protease. Using an in vitro transcription and translation system, we have previously shown that the 37.5 kd preprocathepsin B of *Schistosoma mansoni* is autocatalytically cleaved into an active enzyme of 28 kd. To further characterise its enzymatic activity, we engineered the entire *S. mansoni* cDNA sequence into the genome of the *Autographa californica* nuclear polyhedrosis virus. Both the precursor protein and the mature enzyme are expressed in the lysates of insect cells infected with the recombinant virus, as analysed by western blotting using sera raised in rabbits against the schistosome cathepsin B. In addition, proteolytic activity was demonstrated in extracts containing recombinant cathepsin B but not in extracts of uninfected cells in direct fluorometric assays using peptide subtrates specific for thiol proteases. We are working towards the purification of the recombinant antigen from insect cell lysates. This will help in further elucidating the role of cathepsin B in the parasite's metabolism.

C 326 EXTENSIVE EDITING OF THE CYTOCHROME c OXIDASE III TRANSCRIPT IN THE *HERPETOMONAS* GENUS: THE EVOLUTION OF RNA EDITING, Laura F. Landweber and Walter Gilbert, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

We have identified the gene and transcript for cytochrome oxidase subunit III (COIII) in four species of *Herpetomonas* by direct PCR sequencing of the maxicircle COIII gene and RNA PCR analysis of the edited mRNA. In each of the four species examined, *H. mariadeanei*, *H. samuelpessoae*, *H. muscarum muscarum*, and *H. megaseliae*, nearly 60% of the COIII transcript is created by RNA editing, with the DNA sequence matching the COIII cDNA sequence at every position except for numerous runs of thymidines. The highest degree of sequence conservation is observed at the mRNA and protein level, but there has been sequence compression up to 40% and drift at the DNA level. The low levels of protein similarity, however, in some comparisons are probably due to frameshift mutations that are compensated by editing at another site, as well as a high ratio of replacement to silent nucleic acid substitutions. A peculiar feature of the editing in the COIII gene has been that the *L. tarentolae* and *C. fasciculata* transcripts are edited only at the 5′ end, whereas the *T. brucei* transcript is edited along its entire length. Editing of the COIII transcript in *Herpetomonas* also occurs along its entire length. This monogenetic genus, may be older than the more advanced digenetic species, *T. brucei* and *L. tarentolae*, which would suggest that extensive editing of the COIII gene is an ancient phenomenon that was recently lost in *L. tarentolae*.

C 327 STUDIES ON IMMUNODOMINANT ANTIGENS AND MUSCLE PROTEINS IN *SCHISTOSOMA MANSONI*

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The close relationship between myosin and paramyosin and their related functions in the composition of the muscle fiber of invertebrates prompted us to apply molecular biology techniques to the study of these molecules in schistosomes. A differential screen of a cDNA library identified partial clones, displaying significant homologies of both gene products to sequences obtained from *Caenorhabditis*. Affinity-purified antibodies against the expressed gene products were employed to study the distribution of the proteins histologically.

Anti-myosin antibodies reacted with the more pronounced muscle layer of the male worm, but did also detect the musculature of the female. Correspondingly, Western blots normalized to equal amounts of protein showed an enrichment of myosin in the male. In principle, the same was true for Northern blots but the ratio of mRNA between the sexes was higher than expected, possible explanations for which are currently under investigation.

Although myosin, as expected, was restricted to the muscle layer of adult worms of both sexes, paramyosin, on the contrary, was predominantly localized in the tegument of male and female worms. The low level of staining in the muscle layer, as well as complementary findings of other groups indicate that paramyosin might serve a non-muscle function in schistosomes. Preliminary evidence suggests the existence of isoforms, which might explain the unexpected distribution of paramyosin in the tissue sections. Studies on the mechanism of its well-known immune-dominance in the infected host are under way.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (KU 282/13-1).

C 328 ANTIBODY BINDING TO AND INHIBITION OF SECRETED ENZYMES OF *DICTYOCAULUS VIVIPARUS*: Jacqueline B. McKeand, **David P. Knox, James L. Duncan and *Malcolm W. Kennedy, Department of Veterinary Parasitology and *WLEP, University of Glasgow. and **Department of Biochemistry, Moredun Research Institute, Edinburgh.

Dictyocaulus viviparus, the pulmonary nematode of cattle, is an important cause of morbidity and mortality in many temperate areas. Good immunity is induced post infection and a successful vaccine comprising gamma-irradiated infective 3rd stage larvae has been used for thirty years. Over this period, there has been a distinct lack of interest in this parasite and the aims of this work were to identify important protective antigens present in the various stages of this nematode and to investigate parasite:host relationships in this unusual host environment. Adult parasites cultured *in vitro* produce large quantities of excretory/secretory (E/S) products which, in various guinea pig strains, induce up to 85% protection to reinfection. The E/S contains a heterogenous mixture of polypeptides as shown by radio-iodination and SDS-PAGE. Studies indicate that these ES products contain distinct enzyme activities which were characterised according to substrate and inhibitor specificities. The enzymes include various proteinases and esterases, including acetylcholine esterase (AChE). Immunoglobulin from infected and vaccinated definitive and laboratory hosts binds to and/or inhibits these enzymes. Secretory AChE's were purified by affinity chromatography to permit further characterisation of these enzymes and the host immune responses to them. The information gained from this study provides us with interesting insights into both the induction of protective immunisation with secretory products and the interactions between the host and parasite in this unusual location.

C 329 MOLECULAR ANALYSIS OF *ASCARIS* SECRETED/EXCRETED ANTIGENS: Joyce Moore, Karen J. McCurrach and Malcolm W. Kennedy. Wellcome Laboratories of Experimental Parasitology, University of Glasgow, Glasgow, G61 1QH, Scotland, UK.

The outcome of a parasite infection is influenced by two general factors. On the one hand, the genetic constitution of the parasite determines the range and nature of antigens to which the host will be exposed. Of particular importance in this respect are the antigens positioned at the host-parasite interface, namely surface and excreted/secreted (ES) antigens. On the other hand, the genetic constitution of the host determines the nature and specificity of the immune response elicited by infection. A molecular study of the ES antigens of the nematode *Ascaris suum*, has been initiated. The first approach has been to use a PCR-based method to clone genes encoding proteases, since it is known that the ES material of both infective stage larvae (L2) and lung stage larvae (L3/4) is rich in these enzymes. The second approach has involved the construction of cDNA expression libraries from L2 and L3/4 stages. Characterisation of these libraries and their screening with antibodies raised against ES material will be described.

C 330 CLONING OF *SCHISTOSOMA MANSONI* ADULT WORM PROTEINS WITH POTENTIAL USE FOR PREPATENT IMMUNODIAGNOSIS, Guilherme Oliveira and Walter M. Kemp, Department of Biology, Texas A&M University, College Station, TX 77843.

An adult worm cDNA library was constructed from adult *S. mansoni* and screened with serum from mice infected for 4 weeks with *S. mansoni*. Seven positive clones were isolated, purified, and partially characterized. Insert sizes ranged from 350 to 1600 base pairs. Five of the seven clones have been partly sequenced and two were completely sequenced. Homologous sequences were sought in GeneBank and EMBL databases. One of the completely sequenced clones presented an almost 80% homology to chicken type 5 actin, but the other 6 clones appear unique. Immune mouse serum from 8 week infections also recognized these 7 purified clones. The clones were probed with a tropomyosin labeled insert, but none of the clones were positive. The reactivity of the fusion protein to immune serum and complete cDNA sequence of the individual clones are currently under investigation.

(Supported by NIH Grant # AI 26505 and ARP Grant # 010366-100)

C 331 BIOSYNTHESIS, GLYCOSYLATION AND SURFACE EXPRESSION OF THE ES ANTIGENS OF *TOXOCARA CANIS* LARVAE, Antony P. Page and Rick M. Maizels, Wellcome Research Centre for Parasitic Infections, Imperial College, London, UK.

The infective larvae of the tissue migratory nematode *Toxocara canis* infect a wide host range including humans. These infective larvae can also survive indefinitely in serum-free tissue culture media, during which they secrete copious quantities of the diagnostically valuable ES antigens. The biosynthesis, glycosylation and routes of surface expression of these antigens were analysed. These antigens are synthesized within 10 minutes, and glycosylated within 30 minutes, but are only released into the culture media after a 24 hour delay. The ES antigens are serine and threonine-rich heavily glycosylated glycoproteins, the lower molecular weight forms being glycosylated via *N*-linkages and the higher molecular weight forms predominantly *O*-linked. The most dominant oligosaccharide associated with these antigens is an *O*-linked trisaccharide with homology to the human blood group H antigen. The sites of antigen synthesis and subsequent routes of release of these antigens were examined by applying ES-specific monoclonal antibodies to ultrathin sections of larvae by immuno-electron microscopy. Various organs were associated with antigen synthesis, including the oesophageal glands and the excretory cell, and it is envisaged that the ES antigens reach the surface of the parasite via the mouth, the excretory pore and through the hypodermis. These antigens were not detected on the parasite surface by conventional immuno-EM, but surface expression was observed by cryo-immuno-EM, being localised in the electron dense "Fuzzy" surface coat. This antigenic surface coat is carbohydrate-rich, negatively charged, has muco-polysaccharide staining characteristics and is very sensitive to dehydration procedures, being sequentially stripped from the surface during the alcohol dehydration procedures. The exposed antigens of *T. canis* are therefore not associated with the cuticular surface of this parasite, but with an extra-cuticular surface coat, which has rapid shedding capabilities.

C 332 A MAJOR SUBSTRATE FOR STAGE-REGULATED TYROSINE PHOSPHORYLATION IN *T. BRUCEI* IS A PROTEIN KINASE. Marilyn Parsons[1], Jeffrey A. Ledbetter[2], Andre Nel[3], Gary Schieven[2], and Steven Kanner[2]. [1]Seattle Biomedical Research Institute, Seattle, WA 98109; [2]Pharmaceutical Research Institute, Bristol-Myers Squibb, Seattle, WA 98121, and [3]Dept. of Medicine, UCLA, Los Angeles, CA 90024.

Tyrosine phosphorylation modulates the function of a number of key regulatory proteins in higher eukaryotes. We previously found a set of tyrosine-phosphorylated bands around 44 kDa in *T. brucei* procyclic forms and stumpy bloodforms, but not in slender bloodforms. We have now generated two monoclonal antibodies which react with these tyrosine-phosphorylated proteins. Each antibody detects a doublet at 44 kDa and a minor band at 46 kDa. Immunoprecipitations of ^{32}P-labelled cells reveals that the major phosphoaminoacid in the 44 and 46 kDa species is phosphoserine, but that approximately 5% is phosphotyrosine. Immunokinase assays and renaturation of protein kinase activity following immunoprecipitation and SDS-PAGE demonstrates that both the 44 and 46 kDa proteins are themselves protein kinases. The features of these kinases are similar to those of the MAP2 kinases (*erks*) of mammalian cells, which are important in transducing signals from growth factor receptors. The 44/46 kDa substrate/kinases are found in slender bloodforms as well as stumpy forms. These data suggest that there is a family of related protein kinases which are constitutively expressed, but which are differentially tyrosine-phosphorylated during the trypanosome life cycle. Thus tyrosine-phosphorylation has the potential of modulating key downstream events mediated by these protein kinases at a critical point in the life cycle.

C 333 STRUCTURE & IMMUNE RESPONSE TO A SURFACE-ASSOCIATED POLYPROTEIN OF FILARIA,

William A. Paxton[1], Maria Yazdanbakhsh[2], Susan Tweedie[1], Lisa Ingram[1], Yvonne Kruize[2], Larry McReynolds[3] and Murray E. Selkirk[1] [1]Dept. of Biochemistry, Imperial College, London SW7 2AZ; [2]Dept. of Parasitology, University of Leiden, Netherlands; [3]New England Biolabs, Beverly, MA 01915-5510.

The gene encoding a protein of *Brugia pahangi* which had previously been defined by surface labelling has been cloned and characterised. The protein, termed p15/200, is constitutively expressed in all life-cycle stages, and is composed predominantly of approximately 20 tandemly-repeated domains of 15 kDa. It is synthesised as a large (over 400 kDa) precursor which is progressively processed into the repeat domains which presumably constitute the functional mature product. Gene segments encoding these domains have been cloned via PCR from *B. malayi* and *Wuchereria bancrofti*, and sequence analysis indicates complete and considerable homology respectively between species. Although no function can be inferred from database searches, it is homologous to the predominant protein in the body fluid of *Ascaris*, a molecule which has been implicated as a major allergen. Expression of p15/200 in *E. coli* has confirmed that it is allergenic in Indonesian filariasis patients, and human T cell lines and clones have been generated to it which demonstrate a TH2 profile of cytokine release; ie. High IL4 and no IFNγ.

C 334 A SURFACE PROTEIN OF *FASCIOLA HEPATICA* EXHIBITS HOMOLOGY TO THIOREDOXIN. A.C. Rice-Ficht* and C.D. Richardson. Department of Medical Biochemistry and Genetics, College of Medicine, Texas A&M University, College Station, Texas 77843.

Fasciola hepatica, a hermaphroditic trematode which primarily infects sheep, cattle, and humans, produces a surface antigen which exhibits homology with the active site of the protein thioredoxin. Thioredoxin is a small protein (12kDa) involved in oxidation-reduction reactions and is highly conserved throughout evolution. A clone (FH2020) representing approximately half of this surface protein has been isolated from a cDNA library with rabbit antiserum directed against the tegument of the liver fluke. Antiserum from a rabbit immunized with recombinant protein recognized a native *Fasciola* protein of approximately 31 kDa. Immunohistochemical studies with the same antisera confirmed the location of the protein to the tegument covering surface spines. Sequence data of the *Fasciola* gene revealed a high homology with the active site of thioredoxin. The Cys-Gly-Pro-Cys sequence of the active site has also been documented in other proteins and classes of proteins involved in protein folding and posttranslational processing.

C 335 P.FALCIPARUM UNDERGOES CLONAL ANTIGENIC VARIATION IN VITRO. David J. Roberts, Gerrad Nash*, Alister G.Craig, Anthony R.Berendt, Kevin Marsh, Christopher I. Newbold, Molecular Parasitology Group, Institute of Molecular Medcine, John Radcliffe Hospital, Oxford OX3 9DU. UK. and *Dept of Haematology, University of Birmingham, Birmingham. B15 2TT. UK.

Clonal antigenic variation in parasite derived red cell surface antigens has been demonstrated for many Plasmodium sp. *(P. knowelsi, P. fragile, and P. chabaudi)*. The wide serological diversity of these antigens for *P. falciparum* suggests this may also be true for the human parasite. We have investigated the potential for clonal antigenic variation in *P. falciparum* by examining the phenotype of cloned lines derived by micromanipulation.

A endothelial binding line was cloned by micromanipulation. Four clones were obtained. Three of these clones had a similar antigenic type to each other and the parental line. One of these antigenical similar clones was recloned by micromanipulation yielding 20 subclones. Approximatley half of these subclones expressed neoantigens at the PRBC surface indistinguishable from the parental clone. However the remaining clones expressed different neoantigens at the PRBC surface from the parental clone. At least 5 different antigenic types exist among these non-parental clones. Functional studies revealed that these 5 antigenically variant clones showed similar binding to CD 36 but greatly reduced binding to ICAM-1-Fc when compared with the parental clone. Contamination of cultures was eliminated by the use of polymorphic DNA probes and PFGE.

We conclude that clonal antigenic variation in *P. falciparum* takes place rapidly in vitro in the absence of selection, a clone can give rise to more than one variant type and provide strong evidence that the expession of variant antigen is associated with the cytoadherence phenotype.

C 336 CLONING AND CHARACTERIZATION OF A *PLASMODIUM FALCIPARUM* CYSTEINE PROTEINASE GENE. Philip J. Rosenthal, Fidel Salas, Jenny Fichmann, and Richard G. Nelson, Parasitology Laboratory, Depts. of Medicine and Pharmaceutical Chemistry, San Francisco General Hospital and U. California, San Francisco, CA 94143.

Intraerythrocytic malarial trophozoites degrade hemoglobin to provide free amino acids for parasite protein synthesis. Hemoglobin degradation is blocked by inhibitors of the cysteine class of proteinases, suggesting that a cysteine proteinase is required for this process. We previously identified an Mr 28,000 cathepsin L-like cysteine proteinase of *P. falciparum* trophozoites (TCP) as a putative hemoglobinase. To identify the gene encoding TCP, we performed PCR with *P. falciparum* genomic DNA and degenerate oligonucleotide primers based on sequences of cathepsin L that are well conserved among papain-family proteinases and amplified a 549 base pair cysteine proteinase gene fragment. The fragment was used as a probe to isolate a 1.8 kb clone from a *P. falciparum* genomic DNA library. The clone encoded a 68.8 kD protein that contained a typical hydrophobic signal sequence, a large pro sequence, and a 26.9 kD mature proteinase that had 37% amino acid identity with cathepsin L. Fragments amplified from genomic DNA and reverse transcribed RNA were of identical size, indicating that the gene contains no introns. In Northern blots, the clone hybridized most strongly with RNA from rings, the life-cycle stage immediately preceding trophozites. The portion of the 1.8 kb clone predicted to encode mature TCP was subcloned into a pET (T7 promoter) expression vector. *E. coli* transformed with this vector produced an abundant Mr 32,000 recombinant protein. In immunoblots, both antisera directed against the recombinant protein and antisera directed against a peptide encoded by the 1.8 kb clone recognized an Mr 28,000 trophozoite protein. Our results suggest that the proteinase gene we have characterized encodes TCP.

C 337 ISOLATION OF A SPECIFIC RECOMBINANT *DICTYOCAULUS VIVIPARUS* ANTIGEN WITH IMMUNODIAGNOSTIC POTENTIAL. Thomas Schnieder, Institute of Parasitology, School of Veterinary Medicine, W-3000 Hannover 71, FRG

A species specific antigen of adult *D. viviparus* has been identified and characterized by SDS-PAGE and immunoblotting analysis. Rabbits have been immunized with a gel cut-out of the specific antigen to produce a rabbit anti-*Dictyocaulus* serum. A λ ZAP II cDNA library has been constructed from *D. viviparus* adult worm mRNA generating 4.4 x 10^5 recombinant primary clones (representing 88 % of the total number of clones). After immunoscreening of the *D. viviparus* adult worm cDNA library with the rabbit immunserum, eight positive clones have been picked and allocated to the same antigenic family by sibling analysis. The recombinant clones have been subcloned into the plasmid vector pGEX-2T and expressed in *E. coli* as glutathion S-transferase (GST) fusion protein or, after cleavage with thrombin, as pure recombinant parasite protein. The clone with the highest expression yields has been selected for further investigations. The native parasite antigen coded for by the clone has been identified as a M_r 18,000 stage specific adult worm antigen. The immunodiagnostic potential of the recombinant proteins has been assessed by immunoblotting and enzyme-linked immunosorbent assay. In immunoblots the thrombin cleaved recombinant protein has been recognized by 100 % of the *Dictyocaulus* positive sera and not by negative sera or sera from other nematode infections. In the ELISA only positive sera have shown high absorbance values. Sensitivity and specificity have been calculated as 93.5 % / 93.5 % for the thrombin cleaved pure recombinant protein and 87.1 % / 96.6 % for the GST fusion protein. The recombinant antigen will be assessed for its protective potential.

C 338 CUTICLE STRUCTURE AND ANTI-OXIDANT ENZYMES OF LYMPHATIC FILARIAL PARASITES, Murray E. Selkirk, Edith Cookson and Liang Tang, Department of Biochemistry, Imperial College of Science & Technology, London SW7 2AZ, England.

The nematode cuticle is an extracellular matrix which acts as an exoskeleton, but also represents the main body of tissue in contact with the immune system of the mammalian host. It is composed predominantly of 2 classes of highly cross-linked structural proteins, collagens and 'cuticlins'. In addition, a small number of soluble proteins with a degree of stage- or species-specificity can be identified by surface labelling. We have recently cloned and identified the major soluble cuticular protein of adult *Brugia pahangi, B. malayi* and *Wuchereria bancrofti* as a secretory form of glutathione peroxidase (GSHPx) which shows approximately 43% homology to mammalian cytosolic enzymes. Subcloning of full length cDNA into an SP6 vector and subsequent translation in vitro indicates that the clone represents a true secretory protein, as it is translocated into pancreatic ER preparations and glycosylated following cleavage of the signal peptide. Other differences with mammalian GSHPx include the presence at the active site of a UGC codon rather than the UGA which typifies selenocysteine. Selenium is incorporated into the protein, however, suggestive of a novel mechanism for selenocysteine synthesis. Possible biological functions for such a secretory enzyme in filariae include inhibition of the oxidative burst of host leucocytes, potentially limiting cytotoxic effector mechanisms. Another possibility is that the enzyme catalyses the formation of tyrosine-derived cross-links known to exist in nematode cuticular proteins, and we are attempting to address these two alternatives. Genes for another anti-oxidant, superoxide dismutase, have been isolated, and we are raising antisera to see if this enzyme co-localises with GSHPx in the cuticle.

C 339 CLONING AND CHARACTERIZATION OF *TRYPANOSOMA CRUZI* ANTIGENS THAT SHARE HOMOLOGY WITH HUMAN RIBOSOMAL P-PROTEINS. Yasir A.W. Skeiky, Darin R. Benson, Marilyn Parsons and Steve G. Reed. Seattle Biomedical Research Institute, Seattle, WA. USA.

We have cloned and characterized two full length cDNAs from *Trypanosoma cruzi* encoding proteins TcP0 and TcP2 homologous to the human ribosomal phosphoproteins HuP0 and HuP2. TcP0 is transcribed as a ~1.3 Kb message and expressed as a 45 kDa protein at all stages of the life cycle. Both TcP0 and TcP2 are present in multiple copies. The *T. cruzi* P- proteins show a clustering of residues that are evolutionarily conserved in higher eukaryotes. This includes a Ala-,Gly-,Pro- rich region adjacent to a highly charged C terminus. This domain is the "hallmark" of the eukaryotic P-protein family and is the basis of their immunological cross-reactivity. However, the C-terminal 11 amino acids of TcP0 shows a variation from that of HuP0 while TcP2 is more homologous to HuP2. A subset of patients with systemic lupus erythematosus (SLE) possess autoreactive antibodies directed against the ribosomal P-protein family. We found that all Chagas' patients tested had antibodies reacting with recombinant TcP0. Deletion of the six carboxyl-terminal amino acids abolishes the reactivity of Chagas' sera with TcP0. In addition, sera from SLE patients that reacted with human P-proteins also showed reaction with TcP0 as well as with *T. cruzi* proteins with MW characteristic of TcP0, TcP1 and TcP2. *T. cruzi* P- proteins may therefore contribute to the development of autoreactive antibodies. This work supported by NIH grants AI-22726 and AI-16282. Y. Skeiky is a fellow of the Medical Research Council of Canada.

C 340 CHARACTERIZATION OF A cDNA ENCODING THE GP63 MAJOR SURFACE PROTEIN OF LEISHMANIA GUYANENSIS. Holly B. Steinkraus and Pamela J. Langer, Department of Molecular Biology, University of Wyoming, Laramie, WY 82071. Data have suggested that gp63 is highly conserved among diverse Leishmania species. To analyze gp63 gene homologues in the L. braziliensis complex, a full length L. guyanensis stationary phase promastigote cDNA clone was isolated and sequenced in its entirety. The predicted sequence of the mature L. guyanensis gp63 is somewhat longer than previously reported gp63 sequences, containing 523 amino acids as compared with 502 in L. chagasi and in L. major. Although there is a high sequence identity between L. major and L. chagasi (88%), the L. guyanensis gp63 sequence shares only 73% sequence identity with L. chagasi and 74% with L. major on the DNA level. On the amino acid level, these homologies drop to 64% and 66%, respectively. L. guyanensis gp63 possesses a putative signal sequence cleavage site between Ala-37 and Gln-38, a potential propeptide cleavage site between Val-98 and Ala-99, and a putative zinc binding region between Ala-256 and Thr-273. Northern blot analysis of total RNA revealed a broad band of hybridizing RNA ranging in size from approximately 2.6 to 3.0 kB suggesting substantial gp63 gene transcript size heterogeneity in L. guyanensis stationary phase promastigotes. A minor transcript of 6.1 kb was also detected at approximately 1% the level of the collective major transcripts. Gp63 sequence heterogeneity in L. guyanensis is presently being examined. We have identified at least five unique L. guyanensis stationary phase promastigote gp63 cDNA clones sharing as little as 62% sequence identity with each other. One clone in particular is more similar to L. chagasi gp63 than to any other L. guyanensis gp63 cDNA clone.

C 341 THE INFLUENCE OF MHC HAPLOTYPE IN IMMUNE LIVER TRAPPING OF *TOXOCARA CANIS*. V.A. Stewart, J.C. Parsons, and R.B. Grieve, Department of Pathology, Colorado State University, Fort Collins, CO 80523

Toxocara canis embryonated eggs can infect a number of paratenic hosts, and the larvae, after penetrating the intestine and passing through the portal system, usually disseminate widely in various tissues. A specific immune response generated by primary exposure to live T. canis larvae results in a proportion of larvae from subsequent exposure being retained within the liver. The proportion of larvae retained varies with the inbred mouse strain. C57Bl/6 and C57Bl/10 strains of mice, both H-2^b, retain large numbers of larvae in the liver upon second exposure (challenge). DBA/2 and BALB/c mice, both H-2^d, retain relatively few larvae. Other strains are intermediate. MHC recombinant strains of mice were compared with parent strains of both low and high trapping response phenotype to determine the relative involvement of the MHC loci in the phenomenon of immune liver trapping. The influence of MHC genes was found to be minimal compared to the influence of the genetic background of the strains examined. (Supported by a grant from the College Research Council, College of Veterinary Medicine and Biomedical Sciences, Colorado State University.)

C 342 A SEREPIDEMIOLOGICAL STUDY OF ONCHOCERCA VOLVULOUS IN ECUADOR USING SPECIFIC RECOMBINANT ANTIGENS, Katharine R. Trenholme, Andrew J. Gillespie, Timothy I. M. Tree, Ron Guidenia and Jan E. Bradley, Department of Biology, Imperial College, Prince Consort Road, London SW7 2BB U.K.

Three recombinant *Onchocerca volvulus* antigens (OVMBP10, OVMBP 11, OVMBP 29) were selected for their specificity and sensitivity, primarily for use as diagnostic probes. However the availability of these defined antigens also creates new opportunities for exploring the seroepidemiology of *O.volvulous* infections. The prevalence of IgG antibody subclasses to the 3 antigens, used individually and in combination, was determined using an Enzyme Linked Immunosorbent Assay (ELISA) in microfilaremic and amicrofilaremic individuals from areas of Ecuador with high transmission rates of *O.volvulus* . The factors influencing immune responses to these antigens was examined and differences in responsiveness between racially distinct groups was observed.

C 343 SELECTIVE SCREENING AND CLONING OF ANTIGENS OF Onchocerca volvulus LARVAE. Christopher B.Tume[1], Eugene Sun[2], Gabriel Lando[1], Ian Bathurst[3], Philip J. Barr [3], James H. McKerrow[2] and Jacob L. Ngu[1].

1. Immunology service, CUSS, University of Yaounde Cameroon.
2. Dept. of Pathology, University of California San Francisco, School of Medicine, CA 94143-0506.
3. Molecular Biology Dept., Chiron Corporation, Emeryville, California 94608-2916

Monoclonal antibodies (Mabs) were produced against somatic antigens of Onchocerca volvulus infective larvae (L_3) and extensively characterised vis a vis their IgG subclasses, antigen localisation on worm cross sections, apparent binding affinities to their respective epitopes, Western blot analysis,biochemical nature of epitopes eliciting their production, and their abilities to mediate antibody-dependent mechanisms of leucocyte adherence and cytotoxicity to microfilarial targets. These Mabs were then used to immunoscreen O. volvulus adult and L_3 cDNA libraries to isolate DNA fragments of interest. These were subcloned and sequenced to allow identification of predicted gene products. Clones of interest as potential vaccine components or as key functional parasite proteins were subcloned into a yeast expression system for large scale production.

C 344 SECRETORY ACETYLCHOLINESTERASES OF *NIPPOSTRONGYLUS BRASILIENSIS*, Susan A. R. Tweedie, Michael E. Grigg, C. Clare Blackburn and Murray E. Selkirk, Department of Biochemistry, Imperial College of Science & Technology, London SW7 2AZ, England.

Many nematode parasites of the gastrointestinal tract secrete enzymes which have been defined as acetylcholinesterases (AChEs). *Nippostrongylus brasiliensis* secretes multiple electrophoretically distinguishable forms of AChE, which can be resolved into 2 major classes via their affinity for enzyme inhibitors, and are expressed differentially during the life cycle. We have purified secretory AChEs from the adult stage of this parasite and raised polyclonal and monoclonal antibodies in order to address the relationship between the enzyme isoforms. These data suggest that there are 2 active subunits, with molecular weights of 74 kDa and 39 kDa. In vitro translations, metabolic- and active-site labelling and peptide mapping demonstrate that the AChEs derive from a single primary translation product of 59 kDa which is glycosylated to produce the higher molecular weight subunit, which is in turn processed differentially during the life cycle resulting in the smaller protein. Following secretion, a proportion of the enzyme becomes bound to the cuticular surface, as evidenced by immunoelecton microscopy and surface-labelling studies. We are now attempting to isolate cDNA clones in order to investigate the structure, processing and physiological activity of these enzymes.

C 345 CLONING OF ANTIGENS SHARED BETWEEN *SCHISTOSOMA MANSONI* AND ITS INTERMEDIATE HOST *BIOMPHALARIA GLABRATA*, David S. Weston and W. M. Kemp, Department of Biology, Texas A&M University, College Station, Texas 77843

Schistosoma mansoni and its intermediate host *Biomphalaria glabrata* share antigenic epitopes but the degree of similarity between these shared antigen molecules is unknown. In an attempt to further characterize these shared antigens an adult *S. mansoni* Lambda ZAP expression library was constructed and screened with rabbit anti-uninfected snail hepatopancreas homogenate. Out of 1.8×10^5 recombinants screened, 34 positives were observed. Differential hybridizations performed on these positives defined three distinct subpopulations, one of which specifically hybridized to the schistosome tropomyosin clone, pSMTM [Xu et al. (1989) Exp. Parasit., 69:373]. DNA sequence analysis identified the remaining two categories as schistosome myosin heavy chain (pSMmhc), and a second schistosome tropomyosin isoform (pSMTMB). A *B. glabrata* cDNA library was constructed and screened with the pSMTMB insert at low stringency yielding a snail tropomyosin isoform (pBGTMB) different from the tropomyosin isoform (BG39) [Dissous et al. (1990) Mol. Biol. Parasit. 43:245]. The two snail tropomyosin isoforms have very high (97%) amino acid homology with one another, the difference being due to a region corresponding to a.a. 189-213. In other organisms this region corresponds to exon 6 which undergoes alternative splicing. This putative exon gives pBGTMB higher (81%) homology with pSMTM than BG39 has with pSMTM (75%). Whether this is due to molecular mimicry or conserved housekeeping function is under investigation. (Supported by Office of Naval Research; NIH #AI-26505; Texas Advanced Research Program #010366-100)

C 346 CLONING AND SEQUENCING OF THE 93 KDA *PLASMODIUM CHABAUDI* ACIDIC PHOSPHOPROTEIN, Mark F. Wiser[1], Gregory J. Jennings[1], Willy Deleersnijder[2] and Jean M. Lockyer[3], Department of Tropical Medicine[1] and Human Genetics Program[3], Tulane University Medical Center, New Orleans, LA 70112 and Vrije Universiteit Brussel[2], Instituut voor Moleculaire Biologie, Belgium.

A 93 kDa *P. chabaudi* protein, referred to as Pc(em)93, that interacts with the cytoplasmic face of the erythrocyte membrane has been previously characterized (1). A recombinant lambda gt11 expressing a protein recognized by a monoclonal antibody (Mab-13.5) against Pc(em)93 was isolated. The 2.3 kb insert was subcloned into pBluescript and sequenced by preparing nested deletions. The cloned gene contains a block of 75 nucleotides that is repeated 7 times in tandem followed by a very similar block of 72 nucleotides (one codon missing) repeated twice. Mab-43, prepared against the recombinant protein, also recognizes a protein referred to as Pch105/RESA (2) which also is equivalent to a protein referred to as Pc96 (3) suggesting that all three of these *P. chabaudi* proteins are the same. Although immunofluorescence using Mab-13.5 and glutaraldehyde fixed cells results in a fluorescence of the erythrocyte membrane similar to RESA/Pf155, Pc(em)93 is distinct from RESA/Pf155. In contrast to RESA/Pf155, Pc(em)93 is synthesized by the ring-stage parasite and rapidly transported to the erythrocyte membrane. In addition Pc(em)93 persists on the erythrocyte membrane throughout the intraerythrocytic cycle.

1. Wiser *et al.*, Mol. Biochem. Parasit. 27,11 (1988)
2. Holmquist *et al.*, Exp. Parasitol. 70,436 (1990)
3. Wanidworanun *et al.* Mol. Biochem. Parasit. 25,195 (1987)

Parasite Biochemistry, Genome Organization and Host-Parasite Interactions - II

C 400 EXPRESSION CLONING OF A DEVELOPMENTALLY REGULATED SURFACE PROTEIN OF *Eimeria bovis*. Mitchell Abrahamsen, Rhonda Tintzman, Mark A. Jutila, C.A. Speer and Michael White, Veterinary Molecular Biology, Montana State University, Bozeman, MT 59717

A cDNA clone from partially sporulated oocysts of *Eimeria bovis* was isolated using monoclonal antibody (2.4) generated against *E. bovis* sporozoites. Immunoblotting demonstrated that 2.4 recognizes multiple surface antigens of sporozoites which are absent on merozoites. Immunofluorescence analysis by microscopy or flow cytometry of intact *E. bovis* sporozoites and merozoites revealed exclusive and abundant expression of the antigens in the sporozoite stage. Northern blot analysis of sporozoite and merozoite RNA with the identified cDNA clone revealed a single mRNA species whose expression was consistent with the developmental regulation of the surface antigens recognized by 2.4, indicating that the multiple antigens arise from posttranslational modifications of a single protein. DNA sequence analysis revealed an open reading frame of 127 amino acids which shows little homology to any known sequence.

C 401 ACTIVITY OF A DIVERGENT *TRYPANOSOMA BRUCEI* PHOSPHOGLYCERATE KINASE EXPRESSED IN *E. COLI*. Keith Alexander and Marilyn Parsons, Seattle Biomedical Research Institute, 4 Nickerson St., Seattle, WA 98109

The phosphoglycerate kinase (PGK) gene complex of *Trypanosoma brucei* contains three tandemly linked related genes. One gene encodes a cytoplasmic PGK, while another encodes a PGK isozyme localized to glycosomal microbodies. We have recently shown that the 56-kDa protein, encoded by the 5' most gene in this complex, is also localized to the glycosomal core. Interestingly, it lacks the C-terminal extension found in the glycosomal PGK, thought important for targeting to the glycosome. This protein shows amino acid sequence identity with many of the residues, that are believed necessary for PGK activity, although the overall homology with the other two PGKs from *T. brucei* is only ~80%. In addition there is an 80 amino acid insertion near the N-terminal of the 56-kDa protein, which is lacking in other PGKs (*T. brucei*, yeast or mammalian) and is thus of unknown function. We have expressed this protein in *E. coli* both as a fusion protein containing an extra 23 amino acids at the N-terminal and in its native form. Both proteins exhibit PGK activity in the standard enzyme assay (3-phosphoglycerate + ATP to 1,3-diphosphoglycerate + ADP) and both proteins complement a pgk$^-$ strain of *E. coli*. They have been partially purified from *E. coli* extracts by ion exchange chromatography and can be identified by western analysis using polyclonal sera raised against the glycosomal form of PGK.

C 402 CYTOKINE PRODUCTION IN BALB/c MICE IMMUNISED WITH RADIATION ATTENUATED THIRD STAGE LARVAE OF *BRUGIA PAHANGI*. A.Bancroft, E.Devaney, K.Else* and R.Grencis*. Department of Parasitolgy, School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA. *Department of Cell and Structural Biology, Stopford Building, Oxford Road, Manchester M13 9PT.

Radiation attenuated third stage larvae (L_3) of filarial parasites are known to stimulate good protective immunity in a variety of animal models. Much of the earlier work in rodent models suggested that the basis of this immunity was cell mediated. In order to further our understanding of the control of immunity in BALB/c mice immunised with radiation attenuated L_3 of *B.pahangi* we have examined cytokine production in supernatants of spleen cells from control and immune mice. Spleen cells were stimulated with either Con A or adult antigen and levels of IFN-γ and IL-5 were measured. These experiments demonstrated that spleen cells from immunised animals secreted high levels of IL-5 and negligable levels of IFN-γ in response to either stimulus. Further confirmation of the induction of cells of the T_H2 subset was obtained by analysing supernatants for two further T_H2 specific cytokines, IL-4 and IL-9. No difference was observed in the ratio of $CD4^+/CD8^+$ cells in spleens of control and immunised mice when analysed by FACS. The pattern of cytokine secretion cannot, therefore, be explained by alterations in the ratio of T cells from immune mice.

C 403 IN HOT PURSUIT OF THE *Lsh* GENE, Jenefer M. Blackwell, Tamara I.A. Roach, Sara E. Atkinson, James W. Ajioka, C. Howard Barton and Marie-Anne Shaw, Department of Medicine, University of Cambridge Clinical School, Addenbrooke's Hospital, Cambridge UK CB2 2QQ.

The macrophage resistance gene *Lsh/Ity/Bcg*, first described almost two decades ago, regulates the host response to *Leishmania donovani*, *Salmonella typhimurium* and a range of species of *Mycobacterium* including the vaccine strain BCG. Working *in vitro* with resident peritoneal, liver (Kupffer cells) and bone marrow derived macrophages from congenic B10 (*Lsh*^{s}) and B10.*L-Lsh*^{r} mice we have shown that the final effector mechanism for the gene in regulating antileishmanial activity involves production of reactive nitrogen rather than reactive oxygen intermediates. This in turn is dependent upon priming/activation of macrophages for enhanced TNF-α release which acts back on the macrophage in an autocrine manner to increase nitric oxide production. The precise point at which *Lsh* acts to control macrophage priming/activation has not been identified but studies of early response gene expression show differences in KC mRNA levels at 2 hours after LPS stimulation, and in c-fos mRNA as early as 20 minutes after stimulation with PMA plus ionophore, in peritoneal macrophages from congenic *Lsh*^{s} and *Lsh*^{r} mice. Data available suggest that both negative and positive signals may be involved in macrophage priming/activation, with *Lsh*^{s} macrophages down regulating their capacity for continued response to the autocrine loop. Work in progress will examine the role of TPA and cAMP response element binding proteins in regulating gene expression in *Lsh* congenic mice. A major new initiative has also commenced to clone the *Lsh* gene by reverse genetics using yeast artificial chromosomes to walk towards *Lsh* from the closest proximal and distal markers on mouse chromosome 1. Family linkage analysis using restriction fragment length polymorphisms for marker genes on human chromosome 2q has begun to provide some evidence that a human homologue for *Lsh* may regulate resistance and susceptibility to leprosy and tuberculosis in man.

C 404 CHARACTERIZATION OF THE LARGEST SUBUNITS OF *PLASMODIUM FALCIPARUM* RNA POLYMERASE I, II, AND III. David J. Bzik, Wu-Bo Li, Barbara A. Fox, Manami Tanaka, Haoming Gu, and Joseph Inselburg. Department of Microbiology, Dartmouth Medical School, Hanover, NH 03756.

The genes encoding the largest subunits of *Plasmodium falciparum* RNA polymerase I, II, and III were cloned, sequenced, and characterized. Data for the chromosome assignment and for the mRNA expression pattern will be presented. We demonstrate here that the *Plasmodium* subunits are the largest largest RNA polymerase subunits yet characterized from any lower or higher eukaryote. The *Plasmodium* subunits contain remarkable enlarged variable domains that separate conserved domains in these subunits. Analysis of the enlarged variable domains has revealed some interesting amino acid sequences. Although a definate role for these unique enlarged variable domains has not been elucidated, the presence of amino acid motifs and the large size of the variable domains suggest that the variable domains play some novel role in transcription in *Plasmodium*. We will discuss some potential roles for these enlarged variable domains, which include the possibility that they may in part regulate the switch from synthesis of A-type 18S rRNA to C-type 18S rRNA by *Plasmodium* RNA polymerase I that contributes to the development of stage-specific ribosomes in this genus. Elucidating the potential roles of these variable domains remains an interesting biological problem, and we further suggest that these unique RNA polymerase subunit variable domains may be potential targets for development of specific antimalarial drugs.

C 405 IMMUNOCHEMICAL AND BIOCHEMICAL CHARACTERIZATION OF THE GLYCEROPHOSPHATE OXIDASE FROM THE BLOODSTREAM FORM OF *TRYPANOSOMA BRUCEI BRUCEI*., Chaudhuri, Minu, and Hill, George, C., Division of Biomedical Sciences, Molecular Parasitology Training Program, Meharry Medical College, Nashville, TN 37208.

Glycerophosphate oxidase (GPO) system, unique to the bloodstream forms of trypanosomes, consists of two components: a FAD containing sn-glycerol-3-phosphate dehydrogenase and a cyanide-insensitive, SHAM sensitive oxidase. These two components are linked via ubiquinol. Glycerophosphate oxidase has recently been solubilized and partially purified from mitochondria of bloodstream forms of *Trypanosoma brucei brucei*. Further characterization of this enzyme is necessary since due to its absence in the mammalian host, this vital parasite enzyme may be used as a chemotherapeutic target. Cytochemical localization and other biochemical studies of this enzyme suggests that it has some similarity with terminal alternative oxidases present in some plants and higher fungi. We found that monoclonal antibody (AOA) developed against *Sauromatum guttatum* alternative oxidase detects two groups of bands of about 58 kDa and 30 kDa in SDS-PAGE and western blot analysis of total mitochondrial protein from the bloodstream forms. These proteins are apparently absent in the mitochondrial extract of the procyclic forms. Further denaturation with urea reveals that the 58 kDa band may be the dimer of the 30 kDa protein. Polyclonal antisera raised against *Symplocarpus foetidus* (skunk cabbage) alternative oxidase also recognized the same group of bands in the mitochondrial extract of the bloodstream trypanosomes. AOA inhibits the glycerophosphate oxidase activity of the detergent solubilized mitochondria of the bloodstream trypanosomes. The 30 kDa protein is being purified from the detergent extract of the purified mitochondria from the bloodstream trypanosomes by immunoaffinity chromatography using the polyclonal antibodies raised against skunk cabbage alternative oxidase. The immunopurified protein is being used for microsequencing and raising trypanosome GPO-specific monoclonal antibody. The polyclonal antibodies against the skunk cabbage alternative oxidase is also being used to screen a cDNA expression library of the bloodstream trypanosomes. Supported by NIH grant #AI 21159 and USAID contract #DAN5053G.

C 406 IN VIVO INDUCTION OF NITRIC OXIDE BY TNF, LT AND IL-1 HAS IMPLICATIONS FOR MALARIAL IMMUNITY AND PATHOLOGY, Ian A. Clark, Kirk A. Rockett, Karen M. Gray, Elizabeth J. Rockett and William B. Cowden, Department of Biochemistry and Molecular Biology, Australian National University, Canberra, ACT 2601, Australia

TNF and related cytokines have been implicated in cell-mediated immunity and pathophysiology in malaria, but their mechanisms of action are yet to be elucidated. We have examined the ability of TNF, IL-1 and LT (three cytokines increased in human malarial serum) to induce nitric oxide (NO) in the plasma of malaria (P. vinckei)-primed and normal mice. Plasma was exposed to a copper/ cadmium/zinc catalyst to convert nitrate to nitrite, then to Greiss reagent, and the product measured colorimetrically. TNF, IL-1 and LT all increased the generation of products of NO, more so in mice carrying sub-clinical infections than in control animals. In contrast, IFN-γ and IL-6 induced negligible NO products in vivo. N^6-methyl-L-arginine inhibited in vivo generation of these products, demostrating their arginine origin.

NO moves freely across membranes, and has recently been recognized as a major second messenger in excitatory synaptic neurotransmission. Therefore the capacity of TNF, IL-1 and LT to induce NO inside blood vessels allows a new model of human cerebral malaria, in which cytokine-induced NO mingles with NO of neuronal origin, thus interfering with orderly cell-to-cell signalling in the brain. In particular, NO has been shown to inactivate the binding protein of glutamate receptors, thus reducing excitatory synaptic transmission. This proposal explains several key features of human cerebral malaria, such as an intact blood-brain barrier, and the rarity of residual neurological deficits, that are inconsistent with the current models for this disease.

Cytokine-induced generation of NO is also consistent with new data on the susceptibility of both the liver and blood forms of malaria parasite to this mediator.

C 407 PLASMODIUM falciparum-INDUCED MODIFICATIONS OF BAND 3 PROTEIN ARE INVOLVED IN CYTOADHERENCE, Ian Crandall and I.W. Sherman, Department of Biology, University of California, Riverside, Ca., 92521. We developed murine monoclonal Mabs (designated 4A3, 1C4, 2B3, 3E12, 4F4, 3H3, and 5H12) against the surface of live P. falciparum- infected erythrocytes. None of the Mabs reacted (by surface immunofluorescence) with either uninfected red cells or red cells bearing ring-stage parasites. By peptide mapping and immunoblotting these membrane antigens were shown to be modified forms of band 3, and included proteins with Mrs of >240, 85, 65,and 55Kda. These parasite-induced neoantigens, were not found in uninfected erythrocytes, aged red cells, or ring infected erythrocytes. The neoantigens are exposed on the outer surface of the infected red cell since live cells bind antibody, they are trypsin and iodine sensitive, and some of the Mabs (4A3, 1C4 and 3H3) directed against them inhibit cytoadherence in a dose-responsive fashion. The band 3-related neoantigens appear to result from truncation and covalent modification of the native form of the protein. The immunoblot patterns produced by the Mabs allowed assignment of the Mabs to two regions of the membrane spanning region of the band 3 molecule: amino (1C4, 2B3 and 3H3) and carboxyl (4A3, 3E12, 4F4, and 5H12). The location of the epitopes to which the Mabs bind was deduced from the sites of chymotrypsin and predicted calpain cleavage. The epitopes of 1C4 and 3H3 are located on external loop 3 (residues 541-575), whereas the epitopes of 4A3 and 5H12 are located on external loop 7 (residues 807-860). Synthetic peptides corresponding to these regions inhibited adherence of infected red cells to amelanotic melanoma cells.

C 408 THE MITOCHONDRION OF *PLASMODIUM FALCIPARUM* - A TARGET FOR TOPOISOMERASE II-ACTIVE DRUGS. Sandra J. Darkin-Rattray and Thomas C. Rowe, Department of Pharmacology and Therapeutics, University of Florida, Gainesville, Florida 32610.

The mitochondrion of malarial parasites is poorly understood. It has received only scant attention from researchers over the years, primarily because the organelle is atypical and difficult to study by conventional means. However, despite these constraints recent data suggest that possible new chemotherapeutics may be obtained by further investigations of this organelle and its genome(s).

In a preliminary study, the mitochondrion of *P.falciparum* has been investigated as a target for antimalarial drugs and the promise of topoisomerase II-active drugs as a class of novel antimalarial agents has been explored. We have observed that ciprofloxacin, a 4-quinolone drug known to inhibit the bacterial type II topoisomerase causes a selective and dose-dependent loss of the 6kb mitochondrial DNA (mtDNA) from *P.falciparum* (Dd2 : Chloroquine resistant) grown *in vitro*. The loss of mtDNA was associated with a decrease in parasite viability. Ciprofloxacin at 32μg/ml (7.6μM) resulted in a loss of approximately 50% of 6kb DNA, correlating with levels of cytotoxicity achieved with a 48h drug treatment. The effect of this drug on the integrity of the 6kb mtDNA is being tested. Furthermore, the levels and integrity of the second organellar genome, the 35kb DNA, found in *P.falciparum* is also being examined.

Our preliminary findings suggest that the mitochondrion of *P.falciparum* may possess a topoisomerase II enzyme that has potential as a target for novel antimalarial therapeutics.

C 409 INTERACTION BETWEEN MACROPHAGE PROTEIN KINASE C AND THE *LEISHMANIA DONOVANI* LIPOPHOSPHOGLYCAN, Albert Descoteaux, Greg Matlashewski*, and Salvatore J. Turco, Department of Biochemistry, University of Kentucky Medical Center, Lexington, KY 40536, *Institute of Parasitology, McGill University, Ste-Anne de Bellevue, Qc, Canada

The cell surface glycoconjugate lipophosphoglycan (LPG) of *Leishmania* promatigotes is a potent inhibitor of purified protein kinase C (PKC) activity *in vitro*. The present study was undertaken to investigate the effect of LPG on the activation process of PKC in murine bone marrow-derived macrophages (BMM). Activation of PKC is a two-step process: (i) a priming step, which requires the translocation of the enzyme from the cytosol to the membrane, and (ii) binding of diacylglycerol or phorbol esters, which fully activates PKC in the membrane. Association of PKC with the membrane was first compared in untreated, LPG-treated, and *L. donovani*-infected BMM. In response to the calcium ionophore A23187, the kinetics and the extent of phorbol dibutyrate ([^{3}H]PDBu) binding were similar in the three groups. Since [^{3}H]PDBu specifically binds membrane-associated PKC, these results indicate that LPG, either exogenously added or naturally presented, did not interfere with the translocation of PKC to the membrane. PKC activity was then assayed *in situ* in digitonin-permeabilized BMM in the presence of the peptide substrate VRKRTLRRL, which is specific for PKC. Preincubation of BMM with 1 uM LPG did not affect the basal level of VRKRTLRRL phosphorylation. On the other hand, PMA-stimulated phosphorylation of the peptide was reduced by >50%, indicating that LPG inhibited PKC activity. Therefore, the inhibitory effect of LPG on PKC-dependent processes in macrophages may reflect an inhibition of the activity of membrane-associated PKC, inasmuch as the association of the enzyme with the membrane takes place normally. The intracellular growth of *L. donovani* was compared in normal and PKC-depleted BMM. While the initial uptake of parasites was similar for both groups, depletion of PKC resulted in a two-fold increase in the number of amastigotes 48 h post-infection. Therefore, inhibition of PKC-dependent events during the early stage of infection may be critical for the successful growth of *L. donovani* in macrophages.

C 410 THE EXTRACHROMOSOMAL DNAS OF *PLASMODIUM FALCIPARUM*, Jean E. Feagin[1], Malcolm J. Gardner[2], Donald H. Williamson[2], and Robert J.M. Wilson[2], [1]Seattle Biomedical Research Institute, Seattle, WA 98109-1651, [2]National Institute for Medical Research, Mill Hill, London, NW7 1AA, U.K.

Plasmodium falciparum has two extrachromosomal DNAs, a 35 kb circular DNA and a tandemly repeated 6 kb DNA. The 35 kb DNA encodes large and small subunit rDNAs, tRNAs, a ribosomal protein gene, and subunits of RNA polymerase. The rRNAs are duplicated as an inverted repeat which, with the polymerase genes, is more characteristic of plastid than mitochondrial genomes. The function of the 35 kb DNA remains enigmatic; it has been suggested to be part of a bipartite mitochondrial genome. The 6 kb element encodes protein coding genes characteristic of mitochondrial genomes and fragments of rDNA sequence; despite its small size, it is probably (part of) the mitochondrial genome. Cross-hybridization between the two extrachromosomal DNAs appears confined to rDNA sequences. The 35 kb DNA rRNAs are similar to eubacterial rRNAs and appear to be cotranscribed with flanking tRNAs. The rRNA fragments from the 6 kb element are scrambled in order and much smaller in total than expected for rRNAs. Comparison of corresponding rDNA sequences from the 6 kb and 35 kb DNAs show that the regions conserved are core regions common to most rRNAs. The rRNAs from the 6 kb element contain predominantly core sequences. The lack of conservation between the extrachromosomal DNAs outside the rDNA core sequences suggests that they are not closely related. rDNAs from both appear more closely related to *E. coli* rDNAs than to each other, further suggesting the two molecules have no recent common ancestor. The extrachromosomal DNAs appear unlikely to have derived from a single mitochondrial precursor and probably have relatively unrelated functions.

C 411 THE GENOMIC ORGANIZATION AND CHROMOSOMAL LOCALIZATION OF GP63 GENES OF LEISHMANIA GUYANENSIS. Joy M. Greer, Holly B. Steinkraus, and Pamela J. Langer, Department of Molecular Biology, University of Wyoming, Laramie, WY 82071. The major surface protein of Leishmania (gp63) is encoded by multiple gene copies in several Leishmania species. These genes appear to be located on a single chromosome in L. major, L. aethiopica, and members of the L. donovani complex, but are found on more than one chromosome in L. tropica, L. amazonensis, and members of the L. braziliensis complex. Others have reported that many of the gp63 genes are arranged in tandemly linked repeated units in L. major, L. chagasi, and L. mexicana. We have examined the genomic organization of gp63 genes in a cloned strain of L. guyanensis. Restriction enzyme and Southern blot analysis of cosmid clones has revealed at least three non-identical gp63 gene clusters. There are three 3.0 kB Apa I, at least two 4.4 kB Cla I, and four 2.85 kB Bgl II repeat units. Hybridization analysis of these three clusters suggests the presence of at least twelve gp63 genes. The clusters may be contiguous thereby generating a large gp63 gene locus spanning approximately 65 kB. However, a L. guyanensis gp63 gene probe hybridizes to two L. guyanensis chromosomal DNAs of approximately 730 and 680 kB in a ratio of 3:1. Using contour clamped homogeneous electric field gel electrophoresis (CHEF) we are currently determining the chromosomal localization of each of the gp63 gene clusters.

C 412 A MALARIA PARASITE MOLECULE, EBA-175, BINDS TO SIALIC ACID ON ERYTHROCYTES WITH A FINE SPECIFICITY CORRELATING WITH INVASION, JD Haynes, FW Klotz, PA Orlandi, G Reuter, SJ Cohen, R Schauer, RJ Howard, P Palese, and LH Miller, Walter Reed Army Institute of Research, Washington, DC 20307, Christian-Albrechts U., Germany, DNAX Res. Institute, Palo Alto, CA 94304, Mount Sinai School of Medicine, New York, NY 10029, and National Institutes of Health, Bethesda, MD 20892. Plasmodium falciparum malaria parasites can invade sialic acid bearing erythrocytes (E) by means of the 175 kDa E binding antigen (EBA-175). Several approaches were used to establish that the type and linkage of sialic acid on E, and not just its charge, were important. Both binding of EBA-175 to and invasion by parasites into inbred mouse E were inversely correlated with the proportion of 9-O-acetylated sialic acid ($Neu5,9Ac_2$) on the E, without changes in charge. Conversely, both binding and invasion were increased by enzymatic removal of the 9-O-acetyl group to give more N-acetyl neuraminic acid (Neu5Ac). Another approach used the knowledge that Neu5Ac is linked α2,3 and α2,6 on human E. Soluble oligosaccharides with Neu5Ac linked only α2,3 to galactose strongly inhibited binding of EBA-175 to human E. In contrast, similar oligosaccarides with only α2,6 linkages were much less effective, and free Neu5Ac was ineffective. EBA-175 bound tightly enough to glycophorin A on E to allow, after solubilization, their precipitation as a complex by an anti-glycophorin A monoclonal antibody. Purified glycophorin A from human E inhibited both binding and invasion; cleavage of α2,3-linked Neu5Ac from the O-linked tetrasaccharides clustered at the N-terminus of glycophorin A markedly reduced its ability to inhibit both binding and invasion. In conclusion, efficient binding and invasion both require sialic acid without 9-O-acetylation and in an α2,3 linkage to galactose, such as that located on the O-linked tetrasaccharides of glycophorin A.

C 413 KINETIC ANALYSIS OF ANTIGEN SPECIFIC LYMPHOKINE RESPONSES IN RESISTANT AND SUSCEPTIBLE MICE DURING INFECTION WITH *TRYPANOSOMA CRUZI*, DF Hoft, RG Lynch, and LV Kirchhoff, University of Iowa College of Medicine and VA Medical Center, Iowa City, IA 52242.
The Tulahuen strain of *Trypanosoma cruzi* causes higher parasitemias and greater mortality in Balb/c relative to C57Bl/6J mice. The goal of this study was to compare Balb/c and C57Bl/6J lymphokine responses to parasite antigens during *T. cruzi* infection to determine if different cytokine patterns correlate with susceptible and resistant phenotypes. At several time points after initial infection with insect-derived metacyclic trypomastigotes lymph node and spleen cell suspensions were prepared from animals of each murine strain. These lymphocyte suspensions were stimulated with antigen prepared from total parasite lysate and culture supernatants were tested for levels of IFNγ, IL4, IL2 and IL5. Lymphocytes from C57Bl/6J mice produced tenfold more IFNγ than Balb/c lymphocytes. However this differential response occurred only for a limited time immediately preceding peak parasitemias. Balb/c lymphocytes secreted increasing amounts of IL4 with increasing duration of infection. Most culture supernatants derived from C57Bl/6J cell suspensions did not contain detectable levels of IL4. Lymphocytes from both murine strains produced IL2 and IL5 levels not different from uninfected controls. These results indicate that increased numbers or potency of antigen specific IFNγ producing T lymphocytes are present in resistant mice during *T. cruzi* infection. This phenomenon may be responsible for the lower parasitemias and lower mortality seen in C57Bl/6J mice. These data also indicate that IL4 production is a marker for the *T. cruzi* susceptible phenotype. Finally, differential production of IL2 or IL5 was not found, suggesting that these lymphokines are not important factors in *T. cruzi* resistance or susceptibility.

C 414 PROTECTION OF AOTUS MONKEYS FROM MALARIA INFECTION BY RECOMBINANT HYBRID PROTEINS, Erika Hundt, Bernhard Knapp, Burkhard Enders and Hans A. Küpper, *Research Laboratories*, Behringwerke AG, Marburg, F.R.G.
Based on investigations on the malarial blood stage antigens SERP, HRPII and MSAI from Plasmodium falciparum we have expressed two hybrid proteins in E. coli containing selected partial sequences of these antigens. Antibodies raised against both hybrid proteins in rabbits and Aotus monkeys recognize the corresponding P. falciparum polypeptides. In two independent trials using 13 animals we could show that immunization of Aotus monkeys with either of the two hybrid proteins administered in an oil-based well tolerated formulation can protect the animals from an experimental P. falciparum infection.

C 415 STABLE TRANSFORMATION OF TRYPANOSOMA BRUCEI WITH THE PHLEOMYCIN RESISTANCE GENE AND CYCLICAL TRANSMISSION THROUGH THE VECTOR GLOSSINA. Jefferies D., Tebabi P., Pays A., Aerts D., LeRay D., Pays E. Dept of Molecular Biology, University of Brussels, 67 Rue des Chevaux, 1640 Rhode-St-Genese, Belgium.
The neomycin phosphotransferase gene has already been used to stably transform T. brucei by homologous recombination (1,2). Further selectable markers are needed for the genetic analysis of T. brucei, but few have been identified. We report here the stable transformation of T. brucei with a second antibiotic resistance marker, the phleomycin resistance gene (3). A plasmid containing in sequence: the VSG gene promoter (4), the phleomycin resistance gene and the 3' extremity of the tubulin gene locus, was introduced into procyclic trypanosomes by electroporation. Linearisation in the tubulin portion of the plasmid allowed integration into the tubulin locus of the trypanosome genome by homologous recombination. Southern analysis and cloning of genomic fragments showed that insertion was specific for the site of linearisation. Northern analysis and cDNA cloning indicated that the phleo^r gene was transcribed into a 1.3kb RNA which was transspliced and polyadenylated. Analysis of bloodstream transformants generated by cyclical passage through the tsetse fly showed that the inserted plasmid had been retained and that the phleomycin gene was expressed in bloodstream forms. References: (1) Lee & van der Ploeg (1990) Science 250,1583-1587. (2) ten Asbroek et al (1990) Nature 348,174-175. (3) Drocourt et al (1990) NAR 18,4009. (4) Jefferies et al (1991) Mol. Cell. Biol 11,338-343.

C 416 DNA AMPLIFICATION IN α-DIFLUOROMETHYL ORNITHINE RESISTANT *LEISHMANIA*, K. Katakura[1], S. Miyaji[2] and A. Kobayashi[1].
[1] Department of Parasitology, Jikei University School of Medicine, Tokyo105, Japan and
[2] Department of Surgery, Teikyo University School of Medicine, Tokyo173, Japan.

Gene amplification is one of major mechanisms of drug-resistance in *Leishmania*. We selected α-difluoromethylornithine (DFMO)-resistant variants of *Leishmania donovani* by increasing in the drug concentration up to 30 mM in culture. Amplification of extrachromosomal circular DNA molecules was detected in the resistant cells. Size of the amplified DNA appears about 40 kb, although the precise size has not determined yet. The amplified DNA may contain a gene of ornithine decarboxylase (ODC), a target molecule of DFMO. In addition, amplification of linear DNA about 200 kb was found in some variants undergoing DFMO selection. However, occurrence of amplification of the linear DNA was not associated with the degree of DFMO resistance. The amplified linear DNA may be related with small linear DNAs (SLDs) or LD-1 reported previously by others. No cross resistance of the variants was observed with other drugs so far examined and the resistance was not reversed by verapamil.

C 417 ACETYL CHOLINESTERASE HISTOCHEMISTRY OF NERVOUS SYSTEM OF CYSTICERCUS CELLULOSAE, B.V. Ravi Kumar+, S. Vasantha*, S.D. Roopashree+, V. Suryanarayana+ and S.K. Shankar*

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Cysticercus cellulosae (a cestode larva) preferentially infests excitable tissues (muscle & brain) of pigs and humans. Being a multicellular organism it possesses a nervous system (N.S.). We initiated a study on the pathogenesis caused due to interference in host neurotransmission by the parasite *neurotransmitters and neuropeptides*. Towards this we delineated the N.S. of C.cellulosae using Acetylcholine esterase (AchE) histochemistry. The central N.S., situated in scolex, consisted of cerebral ganglia, circumcerebral nerve ring, rostellar nerve ring and its branches. Peripheral N.S., situated in strobila and bladder wall, consisted of a parenchymal (largely motor in function) and a subtegumental (sensory) network. Probably it is also excretomotor and richly innervates excretory ducts. AchE is present in excretory-secretory products of C.cellulosae which may be responsible for morphological changes suggestive of denervation supersensitivity in the porcine muscles. We detected the presence of Adrenocorticotropic hormone like immunoreactivity in peptide extracts of C.cellulosae. We suggest that this neuropeptide may modulate host immune responses.

C 418 A NOVEL EPITOPE FOR PLASMODIUM FALCIPARUM TRANSMISSION BLOCKING VACCINE, Nirbhay Kumar[1], Isabelle Ploton[1], Benjamin Wizel[1], Pietro Alano[2], Richard Carter[2] and Gary Koski[1]. [1]Immunology and Infectious Diseases-SHPH, Johns Hopkins University, Baltimore, MD and [2]University of Edinburgh, Edinburgh.

Transmission of malaria from one infected individual, via the mosquito, to another individual involves the sexual stages of the parasite. Gametocytes and other early developmental stages in the mosquito midgut represent targets for Plasmodium falciparum vaccine. Immunity against these stages which is largely antibody mediated, is known as transmission-blocking immunity (TBI), and operates in the mosquitoes either during fertilization of gametes or during transformation of zygotes into ookinetes. TBI could thus play a central role in reducing transmission and impede selection and spread of vaccine as well as drug-resistant parasites. Target antigens of TBI include gamete surface proteins (230 kDa and 48/45 kDa) produced in the vertebrate gametocyte stages, and a 25 kDa protein produced predominantly after initiation of gametogenesis in the mosquito midgut. The genes for gamete surface antigens have not yet been cloned and the development of a transmission blocking vaccine has been hampered largely by the fact that epitopes recognized by antibodies are reduction-sensitive (conformational in nature). We have identified a non-conformational epitope (designated the C^3-epitope) recognized for the first time by transmission blocking monoclonal antibodies. This linear epitope is shared among 230 kDa, 48/45 kDa, and a 27 kDa protein in the gametocytes, and conserved in various geographical isolates (Wizel and Kumar, PNAS, 1991 In Press). Cloned cDNA for the 27 kDa protein and various fragments generated by the polymerase chain reaction were expressed in E. coli. Using recombinant products and overlapping synthetic peptides we have mapped the C^3-epitope. Mice immunized with one of the immunoreactive fragments developed antibodies capable of reducing infectivity of P. falciparum gametocytes in the mosquitoes. Studies are in progress to produce T cell clones specific for the 27 kDa protein for mapping helper T epitopes in the target antigen. These studies provide a rationale for the development of P. falciparum transmission blocking subunit vaccine based on the C^3-epitope and helper T epitope (s).

(Supported by research grants from the NIH, WHO and John D. & Catherine T. MacArthur foundation)

C 419 PROTEINS TARGETED TO THE HYDROGENOSOME OF TRICHOMONAS VAGINALIS MAY BE DIRECTED BY SHORT AMINO-TERMINAL PRESEQUENCES, Carol J. Lahti, Christine E. d'Oliveira and Patricia J. Johnson, Department of Microbiology and Immunology, Molecular Biology Institute UCLA, Los Angeles, CA 90024

The origin of the hydrogenosome, an unusual organelle found exclusively in certain anaerobic eukaryotes which lack mitochondria, is unknown. It is possible that hydrogenosomes are degenerate mitochondria or that the two organelles arose from a common progenitor. Alternatively, hydrogenosomes may have arisen via endosymbiosis with an anaerobic bacterium. The goal of this research is to examine the biogenesis of this unusual organelle. Our focus of investigation is on targeting and incorporation of proteins into the hydrogenosome of the protozoan parasite, Trichomonas vaginalis. Using genes we have isolated encoding two hydrogenosomal proteins, ferredoxin and the β-subunit of succinyl CoA synthetase (β-SCS), the identification of topogenic signals directing proteins to the hydrogenosome is currently being investigated. We have shown the genes encoding these proteins are synthesized on free polysomes and are thus post-translationally translocated into hydrogenosomes. We have also shown the genes encoding these two hydrogenosomal proteins contain short amino-terminal leader sequences which are absent from the mature proteins isolated from hydrogenosomes. The biochemical nature of these amino-terminal leader sequences are strikingly similar to each other, and to mitochondrial protein presequences. We propose that the amino-terminal leader sequences function as targeting signals for translocation into the hydrogenosomes. To examine this, we have established an in vitro import assay which involves mixing purified hydrogenosomes with radiolabelled ferredoxin and determining if the protein is translocated into the organelle. Successful import is demonstrated by protection of the radiolabelled ferredoxin from added protenases, and cleavage of the amino-terminal leader sequence. Initial import assays show that the radiolabelled ferredoxin does associate with the hydrogenosomes.

C 420 *PLASMODIUM BERGHEI* CIRCUMSPOROZOITE REPEAT-SPECIFIC T CELL CLONE: RECOGNITION OF THE INDUCING CRYPTIC EPITOPE VS. SPOROZOITE NATIVE ANTIGEN. Heidi T. Link and Urszula Krzych. Dept Biology, The Catholic University of America, Dept. Immunology, WRAIR, Washington D. C. 20003.

The molecular context of the priming antigen plays a role in determining the hierarchy of T cell epitopes. The dominant effect that certain sites have over others in addressing T cells has been the subject of many investigations. Epitope mapping studies of Plasmodia circumsporozoite (CS) protein showed a limited number of helper and proliferative T cell sites, but a dominant T cell epitope has not been uncovered. In this study we asked whether cryptic T cell sites could be found on the CS protein and if the corresponding T cells play a role in protective immunity. Overlapping 20-mer peptides, representing the entire CS protein, were emulsified in Complete Freund's Adjuvant and administered peripherally to different mouse strains. Peptide primed lymph node (LN) cells were analyzed 10 to 12 days after immunization for reactivities to the priming and overlapping peptides. Surprisingly, nearly identical cryptic epitopes were mapped in the strains tested, except that only C57Bl/6 ($H\text{-}2^b$) mice responded to the repeat sequence, DPAPPNAN. As expected, DPAPPNAN-induced LN T cells responded to the priming peptides and peptides containing similar motifs, but not to the native sporozoite antigen. After cloning by limiting dilution, a single clone, 14D7, recognized not only the DPAPPNAN peptide, but also the native sporozoite antigen, thus we called it "pseudocryptic" epitope. Although the clone recognized both antigens in a class II restricted manner, it also showed cytolytic activity against target B cells pulsed with sporozoites or the repeat peptide. Lymphokine analysis demonstrated that reactivity with DPAPPNAN peptide induced IL-4, while stimulation with sporozoites induced IL-2. These observations suggest that T cells capable of recognizing the native sporozoite antigens are induced, however, they are not expanded sufficiently upon sporozoite immunization. Moreover, the unique reactivities with the native sporozoites vs the peptide antigen imply that a single T cell clone may perform several immune functions depending upon the signaling mechanism involved in T cell activation.

This work was supported by a WHO Grant.

C 421 DETECTION OF SURFACE ANTIGEN OF TRYPANOSOME CRUZI, López, M.C.; Martin, J. ; Martin, F; Alonso, C., Instituto de Parasitología "López Neyra" . CSIC. Granada - SPAIN

As a new approach to the search for T. cruzi molecules with poten - tial diagnostic or immunoprophylactic value, parasite protein frac - tions were separated and isolated by SDS-PAGE after sonication. The - N-Terminal sequences of eluted and purified proteins was determined - by the Edman-Begg method. These sequences were analyzed for antigeni- city. Structural computerized graphic programs were also used to de - sing synthetic peptides from the isolated proteins.

The ELISA tests performed using the synthetic peptides as antigens, - have shown that some of them reacted specifically with chagasic sera , and that therefore these peptides may be of importance in the study - of T. cruzi antigens.

The S641 peptide that reacted specically with chagasic sera was used to immunize a New Zealand rabbit. The serum showed significant titers of specific antibodies. In immunofluorescence studies the rabbit anti serum recognizes an antigen present in the membrane of air-dried T. cruzi epimastigotes (Tulahuen strain). This result was confirmed by - FACS analysis on epimastigotes. Western blot analysis showed the re - cogniton by the serum of a specific antigen of about 62 kd.

To determine whether this protein represents a surface antigen, anti-S641 antibodies were used to immunoprecipitate ^{125}I-Labelled surface proteins from epimastigote lysates, detecting the 62 kd antigen. In - the other hand a 40-42 kd fragment was obtained when tryponastigote - lysates were immunoprecipitated with the same sera.

An in vitro traslation assay of a T. cruzi poly A± RNA immunoprecipi- tated with the anti-S641 rabbit sera and protein A-agarose revealed - the presence of a specific band of high molecular weight, indicating that the translated protein conserves the antigenic epitope.

C 422 TRANSLATION OF NEMATODE mRNA IN A HOMOLOGOUS CELL FREE SYSTEM, Patricia A. Maroney and Timothy W. Nilsen, Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland OH 44106
Extracts prepared from 32-64 cell *Ascaris lumbricoides* embryos have previously been shown to catalyze transcription by RNA polymerase II and III as well as both *cis* and *trans*-splicing. Here we describe modified whole cell extracts which are active in translation. The S30 extracts are prepared in extremely low salt and show comparable reinitiation activity to *in vitro* translation extracts prepared from mammalian cells. The *Ascaris* extracts were rendered message dependent by micrococcal nuclease treatment and programmed with *Ascaris* polyadenylated RNA. Hybrid arrest experiments with an oligodeoxynucleotide complementary to the 22 nt SL sequence indicate that the majority of mRNAs are *trans*-spliced. These results contrast with previous determinations of nematode *trans*-splicing frequency derived from experiments using rabbit reticulocyte lysates. The underestimate of *trans*-splicing obtained from reticulocyte analyses probably results from the fact that reticulocyte extracts translate trimethylguanosine capped RNAs (*trans*-spliced messages in nematodes possess trimethylguanosine caps) very inefficiently. Our results indicate that *trans*-splicing plays a much more important role in nematode mRNA metabolism than previously suspected.

C 423 NON-CYTOCHROME MEDIATED OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA OF BLOODSTREAM FORM *TRYPANOSOMA BRUCEI BRUCEI*.
Raj K. Maturi, E. Jay Bienen, Georgios Pollakis and Allen B. Clarkson, Jr., Department of Medical and Molecular Parasitology, New York University Medical Center, New York, N.Y. 10016.
The mitochondria of bloodstream forms of *Trypanosoma brucei brucei* are highly active but have a reduced range of function. In contrast to the tsetse-infective procyclic form, they completely lack cytochromes and do not have a functional Krebs cycle. However, the intermediate and short stumpy bloodstream forms, the presumed bloodstream precursor to insect procyclic forms, have a mitochondrial electrochemical potential, an oligomycin-sensitive ATPase, and an NADH dehydrogenase complex (site I of oxidative phosphorylation). These properties suggested the possibility of cytochrome-independent oxidative phosphorylation. We confirmed that the mitochondrial substrate a-ketoglutarate (a-KG) can support respiration (and motility) in the absence of other carbon sources. We find that this a-KG-supported respiration and motility is inhibited by oligomycin indicating that the respiration is tightly coupled to oxidative phosphorylation. This is further supported by the observation that bongkrekic acid (an inhibitor of the mitochondrial adenine nucleotide translocase) also blocks respiration and motility. Neither of these inhibitors interfere with glycerol-3-phosphate supported respiration, as expected. We find that a-KG will maintain a constant whole cell ATP concentration in intermediate and short stumpy forms but the addition of either oligomycin or bongkrekic acid significantly reduces it. Oxidative phosphorylation in the complete absence of cytochromes is a unique observation. Only one of the classic three sites of oxidative phosphorylation is utilized, site 1, and the reduced ubiquinol produced by site 1 is reoxidized by the trypanosome alternative oxidase (TAO). The TAO thus partially substitutes for the function of cytochromes that do not develop until the parasite transforms into a procyclic form.

C 424 CHROMOSOME-SPECIFIC GENETIC MARKERS FOR TRYPANOSOMA BRUCEI, Sara Melville, Justin Sweetman and Richard Le Page, University of Cambridge Department of Pathology, Cambridge CB2 1QP, U.K.
The use of RFLP and isoenzyme markers has established that genetic exchange readily occurs under laboratory conditions between *Trypanosoma brucei* sub-group organisms[1] during the course of their cyclical development in the tsetse fly vector. It is now apparent that trypanosome strains are widely inter-fertile, and that mating is an optional and not an obligatory process in relation to the growth and development of *T. brucei* in the tsetse fly host[2]. However, the actual frequency of mating and recombination in the field remains unknown. When large samples are taken of trypanosome populations from wild caught tsetse flies the genetic markers present appear to reassort in every possible combination[3,4]. The influence of genetic recombination on such aspects of the epidemiology of trypanosomiasis as the origin of new epidemic strains, host-preference, virulence, and the spread of drug-resistant traits needs to be better understood, and this calls for improved methods of strain characterisation. We are therefore developing chromosome-specific genetic markers.
We have analysed the pulse field gel karyotypes of sets of parental and hybrid trypanosomes recovered from laboratory matings, and identified gel bands representing chromosomes in the size range 1-2Mb. Fragments of these chromosomes have been cloned into bacteriophage P1. This library comprises contiguous and overlapping DNA fragments of 75-95kb. Additionally, randomly primed fragments of genomic DNA from the same defined chromosomes have been used to probe a trypanosome cDNA library. cDNA clones which hybridise to the random probes derived from a defined chromosome are being assigned to specific regions of that chromosome within our P1 library. Once sufficient cDNA clones have been isolated and mapped, we shall determine the positions of the cross-over and breakage events which occur during mating in the tsetse fly and estimate recombination frequencies and the physical and map distances between our markers. These studies will then extend to the comparison of marker distributions in field strains. *References:* 1. Jenni L. et al (1986) Nature 322, 173-175. 2. Tait A. & Turner C.M.R. (1990) Parasitology Today 6, 70-75. 3. Gibson W.C.(1990) Parasitology Today 6, 343. 4. Mihok S. et al (1990) Parasitology 100, 219-233

C 425 LEISHMANIA DONOVANI ENHANCES MACROPHAGE VIABILITY IN THE ABSENCE OF EXOGENOUS GROWTH FACTOR, Kathryn J. Moore and Greg Matlashewski, Institute of Parasitology, McGill University, Ste. Anne-de-Bellevue, Quebec, Canada.
Leishmania donovani is an obligate intracellular protozoan which colonizes the macrophage system of its vertebrate host causing visceral leishmaniasis in humans. We routinely use quiescent murine bone marrow derived macrophages (BMM) in our studies on Leishmania-host cell interactions and have consistently observed more BMMs in cultures infected with L. donovani than in control cultures. Normally, BMMs differentiated in the presence of CSF-1 undergo a decline in cell viability of approximately 90% within 24 hours upon removal of this growth factor. We have observed that 5 day old BMMs cultured in CSF-1 containing media and then subsequently infected with L. donovani in the absense of growth factor, exhibited an approximate 50% attenuation of this observed rapid death of the cell. This implies that intracellular infection with L. donovani is able to partially circumvent the death of its host cell in the absence of specific growth factor. This may represent a mechanism in which this parasite enhances its own survival or increases its target cell numbers within the host. We are currently investigating the effects of L. donovani infection on growth factor production in macrophages.

C 426 CHARACTERIZATION OF A *Trypanosoma cruzi* COMPLEMENT BINDING PROTEIN WITH FUNCTIONAL AND GENETIC SIMILARITIES TO THE HUMAN COMPLEMENT REGULATORY PROTEIN, DECAY ACCELERATING FACTOR, Karen A. Norris*, Bonnie Bradt‡ and N. Cooper‡ , *University of Pittsburgh School of Medicine, Pittsburgh, PA 15261 and ‡ Research Institute of Scripps Clinic, La Jolla, CA 92034.

Evasion of destruction by the host complement (C) system is an essential event in the establishment of infection by many microorganisms. In the case of *Trypanosoma cruzi*, lysis of the bloodstream forms requires antigen-specific antibodies and proceeds mainly via the alternative complement pathway (AP). One target for such antibodies is a 160 kDa surface glycoprotein, which we have purified and shown to be a C regulatory protein. Biochemical and genetic analyses of gp160 reveal similarities to human decay accelerating factor (DAF). The *T. cruzi* gp160 protein restricts C activation by binding C3b and inhibiting AP C3 convertase formation and stability. The protein is anchored in the parasite membrane via a glycosyl phosphatidylinositol linkage, similar to DAF. Using anti-gp160 antibodies, we have isolated a portion of the gp160 gene and found that it shares significant DNA sequence homology with the human DAF gene. Anti-gp160 antibodies which mediated C lysis of the parasites were found to inhibit gp160-C3b binding. Antibodies to unrelated *T. cruzi* surface glycoproteins were not able to support C lysis or block C3b binding. Based on these results, we propose that the mechanism of antibody dependent, antigen specific lysis of *T. cruzi* via AP is the result of specific anti-gp160 antibodies which block the C regulatory activity of gp160 at the parasite surface. This allows unrestricted deposition and amplification of C on the parasite surface resulting in lysis.

C 427AN ANTIFOLATE RESISTANCE DETERMINANT ON THE H CIRCLE OF LEISHMANIA, Barbara Papadopoulou, Gaétan Roy and Marc Ouellette, Laboratoire et Service d'Infectiologie, Centre de Recherche du CHUL and Département de Microbiologie, Université Laval, Québec, Canada.

Amplification of H circles, already present in some wild type *Leishmania*, has been described in several *Leishmania* species selected for resistance to unrelated drugs. One of these drugs, the antifolate methotrexate (MTX), is also capable of inducing *de novo* generation of H circles. Vinblastine and arsenite resistant *Leishmania* mutants having amplified H circles were found to be weakly cross resistant to MTX. Taken together these results suggest that a MTX resistance determinant is present on the circle. Fragments of an H circle isolated from a MTX resistant strain were subcloned in a vector suitable for transfection experiments in *Leishmania*. Several subclones, conferring high level MTX resistance were obtained, the smallest having an insert of 4.2 kb. This fragment was also capable of conferring low level cross resistance to trimethoprim and pyrimethamine, two other antifolates. Analysis of the nucleotide sequence of the 4.2 kb fragment is in progress. Two putative genes have already been found to be encoded by this region. One is a homolog of arginine succinate synthetase, an enzyme involved in the penultimate step of arginine biosynthesis. A second open reading frame shows significant similarities with the superfamilly of short-chain alcohol dehydrogenases. This class of enzymes encompasses several different activities in a wide range of organisms, from prostaglandin dehydrogenase in human to polychlorinated biphenyl degradation in *Pseudomonas*. Intituively it is difficult to ascribe a role to either of these two genes in antifolate resistance. Transfection experiments of the isolated genes as well as further sequence analysis are in progress to identify the antifolate resistance determinant. As expected the arginine succinate synthetase was not related to MTX resistance.

C 428 METRONIDAZOLE RESISTANCE IN TRICHOMONAS VAGINALIS, D.V.K. Quon and P.J. Johnson, Department of Microbiology & Immunology and Molecular Biology Institute, UCLA, Los Angeles, CA 90024

Trichomonas vaginalis is a human infective, sexually transmitted, protozoan parasite which is a common cause of vaginitis. Drug resistant strains of trichomonads have been isolated from women who were refractory to treatment with metronidazole, the drug used for treatment of trichomoniasis. Metronidazole is administered as an inactive prodrug and is reduced to its cytotoxic form by the hydrogenosomal protein, ferrodoxin. We have investigated a possible role for *T. vaginalis* ferredoxin in drug resistance. Western blot analysis has shown that the ferrodoxin levels in four drug resistant strains are decreased as compared to a drug sensitive strain. Similarly, Northern blot analysis has revealed that ferrodoxin mRNA levels are reduced by 50% in resistant strains. We have also shown, by nuclear run-on assays, that ferredoxin gene transcription is reduced by 40-60% in resistant strains. The ferrodoxin gene and its 5' upstream region have been sequenced and compared among drug sensitive and resistant strains. Two point mutations, at -178 and -239 nts relative to the start of transcription, have been identified in a resistant strain. Using nuclease protection and gel shift assays, we have shown that a protein of approximately 23 kDa binds to a 28 bp region that encompasses the mutation at -239 nt. The affinity of the protein for this site is reduced in the mutant. These data correlate drug resistance with down regulation of ferredoxin gene transcription and suggest that the observed reduction in intracellular ferredoxin limits the cell's ability to activate metronidazole.

C 429 KARYOTYPE OF DIFFERENT GEOGRAPHIC ISOLATES OF *BABESIA BOVIS* AND *BABESIA BIGEMINA*, *Bimal K. Ray, *Craig W. Bailey, †James B. Jensen and *C. Andrew Carson, *Department of Veterinary Microbiology, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211 and †Department of Microbiology, Brigham Young University, Provo, Utah 84601

Pulsed field gradient gel electrophoresis was used to compare chromosomes of Mexican, Costa Rican and Australian isolates of *B. bovis* and Mexican and Costa Rican isolates of *B. bigemina*. Three molecular species ranging in size from about 1.0 to 3.0 Mb were present in each of the *B. bovis* samples. A fourth chromosome (slightly smaller than 1.0 Mb) was present in the Mexican isolate, which had been passaged continuously in cell culture for nearly 10 years. There were four chromosomes, of approximately 0.7 to 4.5 Mb, in the *B. bigemina* samples. Chromosomal polymorphism appeared to distinguish each *B. bigemina* isolate. *Babesia* species-specific probes were used to confirm the identity and purity of samples.

C 430 COMPARISON OF THE GENE SEQUENCE OF MEMBERS OF THE TSA GENE FAMILY WHICH ENCODE THE 3.4 AND 3.7 KB CLASSES OF mRNAS, Barbara J. Ruef and Jerry E. Manning, Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717

Trypanosoma cruzi expresses a trypomastigote specific surface antigen whose gene, TSA-1, has been isolated and characterized from the Peru strain. TSA-1 is a member of a multigene family (Peterson, et al, 1989) with multiple members being expressed simultaneously within a single parasite (Kahn, et al, 1990). TSA-1 is distinguished from other members of the family by harboring a 27 nt tandem repeat within the coding region of the gene. We have previously identified two classes of Poly A+ RNA of sizes 3.7 and 3.4 kb that share sequence homology with this multigene family (Fouts, et al, 1991). In the Peru strain the 27 nt repeat unit is present only in the 3.7 kb class of RNA. In contrast, recent hybridization studies have revealed the presence of this repeat unit only in the 3.4 kb class of RNA in the Silvio X10 strain. We have determined from Southern blot analysis of total genomic DNA that Silvio contains only one member in the subfamily defined by the 27 nt repeat unit. A Silvio cDNA, TSA-2, has been isolated and its nucleotide sequence determined. TSA-2 is >90% homologous to TSA-1 beginning in the 5' UT region through the putative translational start site and continuing through the 27 nt repeat region with the notable exception of a deletion of the region in TSA-1 corresponding to the signal sequence. The 3' end of TSA-2, from the repeat region through the poly A tail, shares no significant homology with TSA-1. These results suggest the transport and cellular location of proteins encoded by the 3.4 kb class of RNAs may differ from that of the 3.7 kb class of RNAs.

C 431 MEMBRANE TRAFFICKING THROUGH THE PARASITOPHOROUS VACUOLE OF *LEISHMANIA*-INFECTED MACROPHAGES. **David G. Russell and Prasanta Chakraborty.** Department of Molecular Microbiology, Washington University Medical School, St. Louis, MO 63110.

Immunoelectron microscopical characterization of the parasitophorous vacuole of macrophages infected with *Leishmania mexicana* indicates that the vacuole resembles a lysosome with respect to contents and membrane constituents. This presents problems for the parasite, with respect to survival, and the macrophage, regarding maintenance of this compartment.

The parasitophorous vacuole of an established infection (>72hrs) appears to fuse readily with vesicles containing lysosomal hydrolases, but is reluctant to fuse with vesicles containing endocytosed material. Analysis of the trafficking of labelled ligands, both at the fluorescence and EM level, reveals that, although infected macrophages readily endocytose ligands, such as transferrin, *b*-glucuronidase, *a*-2macroglogulin, and fluid phase markers, little material enters the parasitophorous vacuole. In contrast, biotinylation of surface-exposed proteins on infected macrophages shows that constituents of the plasmalemma traffic through the parasite-containing compartment. This suggests that endocytosed material is likely dissociated from its receptor, or degraded prior to mixing of the endocytic network with the parasitophorous vacuole. The results indicate a flow of membrane components through the vacuole and studies with parasite specific antibodies are currently underway to describe egress pathways and their point(s) of intersection with potential antigen presentation pathways.

C 432 GENE EXPRESSION BY BRUGIA MALAYI L2s DURING DEVELOPMENT WITHIN THE INSECT VECTOR, Alan L. Scott and Porwandee Yenburt. Department of Immunology and Infectious Diseases, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD. 21205.

Filarial nematodes undergo dramatic changes during their development within the insect vector. The transformation from a microfilariae to a L2 to a mature infective stage larvae is characterized by striking changes in the morphology of the larvae as well as changes in the rate and number of proteins being synthesized. The goal of this study was to begin to understand the patterns and regulation of protein expression by filarial larvae as the develop within the insect vector with special emphasis on investigating the expression of the antigens associated with the surface of L3 larvae. The results of surface and metabolic labeling experiments indicated that the major B. malayi L3 surface-associated antigens are synthesized by late L2/early L3 parasites and that the synthesis of L3 surface antigens ceases when the larvae exit the vector. An approach utilizing the polymerase chain reaction (PCR) was employed to amplify the mRNA/cDNA being produced by B. malayi larvae obtained after 8 days of development in the mosquito flight muscles. A sub-population of larval message was obtained by amplifying transcripts containing spliced leader (SL) sequences at their 5' ends. The SL-containing sequences were ligated into vectors and the B. malayi L2 SL library was subjected to differential screening to identify genes that may be expressed in a stage-specific fashion. This approach resulted in the cloning of several SL-containing genes that are expressed by all stages of the parasite, such as glyceraldehyde-3-phosphate dehydorgenase, as well as genes that are expressed only by the L2 or L3 stages. One such gene encodes for a 44 kDa protein that is expressed exclusively by B. malayi L2s during development in the insect flight muscles. The method of using PCR to amplify complex populations of mRNA/cDNA obtained from a small number of larval parasites may find a general use in those situations where parasite or other tissues are limited.

C 433 IgG RESPONSES TO LEISHMANIAL AND MYCOBACTERIAL ANTIGENS IN A HUMAN BCG/LEISHMANIA VACCINATION TRIAL, Claire E. Sharples, Marie-Anne Shaw, Marianella Castes and Jenefer M. Blackwell, Department of Medicine, University of Cambridge Clinical School, Addenbrook's HospitaL, Cambridge UK CB2 2QQ and Instituto de Biomedicina, Apartado 4043, Caracas 1010A, Venezuela.

Previous work has shown that immunotherapy (BCG + killed promastigotes) is effective in the treatment of South American cutaneous leishmaniasis. In 1988 a vaccination trial was initiated to study the immune responses of normal volunteers receiving BCG plus killed promastigotes, to determine its potential as a prophylactic vaccine. Initially, 692 volunteers were screened for skin-test reactivity to mycobacterial purified protein derivative (PPD) and leishmanial antigen and a sample of 208 double negative (≤7mm induration) individuals entered into the trial. Four vaccine groups were established to receive: (A) BCG + killed promastigotes; (B) BCG alone; (C) killed promastigotes alone; and (D) placebo. Three vaccine doses were administered at ~8 week intervals. Blood samples were taken for analysis of antibody and T cell responses prior to each vaccination and at a one year follow-up. We report here on the IgG responses of vaccinees to crude leishmanial and mycobacterial antigens, measured by ELISA. Mycobacterial antigens used in ELISA included *Mycobacterium leprae* soluble antigen (MLSA), PPD, and a crude sonicate of BCG. IgG levels to all three showed a significant increase only in groups receiving BCG, which had begun to wane at the one year follow-up. Responses in the placebo group remained at background levels throughout the trial. Interestingly, group C receiving killed promastigotes alone, showed a significant decrease in anti-BCG response to below background levels following the third vaccination. Similarly, all groups showed a reduction in anti-leishmanial antibody responses following vaccination. Studies in progress will examine in more detail both humoral and cellular responses to defined specific (GP63) and cross-reacting (HSP70) antigens.

C 434 CALCIUM REGULATED SECRETION AND MODIFICATION OF HOST CELL ENDOCYTIC COMPARTMENTS BY *TOXOPLASMA*.

L.D. Sibley[1], and J.C. Boothroyd[2]. [1]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, [2]Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA.

Toxoplasma actively invades a wide range of vertebrate host cells where it resides in a modified endocytic compartment that forms by invagination of the host cell plasma membrane. This vacuole resists fusion with other endosomes and lysosomes as shown using confocal immunofluorescence (IFA) to follow the distribution of membrane markers for early endosomes (transferrin receptors), late endosomes (Man6P receptors), and lysosomes (LAMPs). *Toxoplasma*-containing vacuoles in both phagocytic and nonphagocytic cells also remain devoid of the vacuolar H^+/ATPase characteristic of endosomes and lysosomes, suggesting their failure to acidify is due to an exclusion of proton pumps. In contrast, antibody opsonized parasites entering macrophages are rapidly delivered to vacuoles that are H^+/ATPase positive, acidify, and fuse with other endosomes and lysosomes. This model is being used to examine the mechanism whereby *Toxoplasma* enters endocytic vacuoles that resist processing.

To identify unique modifications of this compartment that may explain this resistance to normal processing, we have examined the kinetics and regulation of secretion of GRA2, a major *Toxoplasma* secretory granule protein of 28 kD Mr. Following invasion, GRA2 is rapidly released into the vacuole by regulated exocytosis of secretory granules as determined by confocal IFA and immunoEM. Cell fractionation studies indicate that GRA2 occurs as both a soluble form within the vacuole lumen and as a membrane form that specifically associates with a membraneous network within the modified vacuole. Secretion of both soluble and membrane forms of GRA2 by extracellular *Toxoplasma* cells is inhibited in buffers that simulate extracellular calcium levels (1mM) and stimulated in low calcium buffers (1-10 μM), as monitored microscopically and by quantitative immunoassay. Similarly, secretion of GRA2 by intracellular *Toxoplasma* following invasion is both rapid and extensive within the initial 10 min of vacuole formation. These findings suggest that the free [Ca^{++}] level in the newly formed vacuole may mediate exocytosis of *Toxoplasma* secretory proteins that contribute to modification of the vacuole.

C 435 A RIBONUCLEASE ACTIVITY IN MITOCHONDRIAL EXTRACTS OF LEISHMANIA TARENTOLAE IS ACTIVATED BY DIGESTION WITH PROTEINASE K OR BY HEPARIN, Agda M. Simpson, Norbert Bakalara and Larry Simpson, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

A ribonuclease activity in a 100,000xg supernate of a Triton lysate of a mitochondrial-kinetoplast fraction from Leishmania tarentolae is activated by incubation with heparin or by pre-digestion of the lysate with proteinase k or pronase. In vitro transcribed pre-edited cytochrome b mRNA is cleaved at several sites. With time, complete degradation of the RNA occurs. All cleavages occurred within putative single stranded regions of the RNA. No cleavage was observed with 9S rRNA. The presence of a nonspecific nucleotide or nucleoside slows the rate of cleavage. The cleavage activity is inhibited by SDS or phenol/chloroform extraction, is retained by a 10 kd cutoff filter, and passes through a 30 kd filter. Micrococcal nuclease inhibits the proteinase-induced activity but not the heparin-induced activity. The role of this cleavage activity in RNA processing or turnover is unknown. A possible role of this activity is as a component of the enzymatic machinery for RNA editing.

C 436 LIPOPHOSPHOGLYCAN IS NOT ESSENTIAL FOR VIRULENCE OF *Leishmania major*, Anita C. Skinner, Malcolm J. McConville and Jenefer M. Blackwell, Department of Medicine, University of Cambridge Clinical School, Addenbrooke's Hospital, Cambridge UK CB2 2QQ and Department of Biochemistry, University of Dundee, Dundee UK DD1 4HN.

At 1.25×10^6 copies per promastigote (PM), liphosphoglycan (LPG) is the major surface molecule of *L. major*. It has been proposed as the principal determinant of virulence because (i) it is the major C3 acceptor opsonizing metacyclic PM for entry into macrophages via complement receptors (J Immunol 1989 143:617); (ii) it prevents insertion of the membrane attack complex into the PM membrane (J Exp Med 1988 167:887); (iii) it inhibits protein kinase C (Biochem Biophys Res Comm 1987 148:653) and scavenges reactive oxygen intermediates (PNAS 1989 86:2453); and (iv) it protects PM from degradation within the macrophage (J Immunol 1986 137:3608). To evaluate the importance of LPG in infectivity we have compared the standard *L. major* LV39 strain against a naturally isolated strain (L119, J Trop Med Hyg 1959 62:158) deficient in LPG. 48 clones of L119 were produced by limiting dilution analysis from PM transformed from amastigotes (AM) isolated from footpad lesions 8 months after inoculation of uncloned L119 PM. Ten clones have been analysed for infectivity *in vivo* and *in vitro*, and for ability to bind the lectin PNA and MAbs against common (45D3 and WIC79.3) and metacyclic (3F12) specific epitopes of LPG. None express WIC79.3 or 3F12 epitopes at any stage of the growth cycle. Despite this, 8 are stably virulent and 2 avirulent. Unlike LV39, virulent L119 clones produce large swollen footpads with no necrosis or tissue damage. Two clones bind 45D3, suggesting that its epitope may reside close to the GPI anchor of a truncated LPG, or on the terminal mannose cap common to both LPG and the glycoinositol phospholipids (GIPLs; J Biol Chem 1990 265:7385). 45D3 expression does not correlate with infectivity. Uncloned L119 PM express GIPLs, and we are now analysing PM and AM of the clones in detail for polymorphism in carbohydrate/protein structure and/or expression of GIPLs, GP63 or HSP70.

C 437 A YAC BASED PHYSICAL MAP OF THE UBIQUITIN-CALMODULIN GENE COMPLEX, [1]Swindle, J. and [2]Ajioka, J.W. 1) Dept. of Microbiology and Immunology, University of Tennessee, 858 Madison Ave., Memphis, TN 38163. 2) University of Cambridge Clinical School, Dept. of Medicine, Level5, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK. A yeast artifical chromosome (YAC) cloning system was used to generate a non-rearranged Trypanosoma cruzi genomic library. Due to the repetitive nature of trypanosome genomes most genomic libraries generated using plasmid and phage based cloning systems contain large deletions and/or gene rearrangements, as a result of recombination between members of the tandemly repeated gene families. Therefore it is often difficult to ascertain whether or not a clone of interest represents an accurate reflection of the genomic map. In an attempt to circumvent these problems a T. cruzi genomic library was generated using YACs. High molecular weight genomic DNA was isolated and subjected to partial EcoRI digestion. A 200Kbp to 300Kbp size fraction was isolated and ligated into the EcoRI site of pYAC4 (Burke, D.T., et al., 1987, Science 236:806-812). Saccharomyces cerevisiae strain AB1830 was transformed and ura^+, trp^+ transformants isolated. Two thousand transformants, representing a ten fold library, were picked into an ordered array and screened using a T. cruzi ubiquitin gene probe. Eight clones hybridizing to the ubiquitin probe were isolated and five were analyzed using chromosome gels, Southern blots and polymerase chain reaction. Three of the five clones displayed no rearrangements within a 30Kbp DNA sequence containing the linked ubiquitin and calmodulin genes. This region contains five calmodulin genes, five ubiquitin-fusion genes and five polyubiquitin genes representing a total of approximately fifty copies of the ubiquitin coding sequence. Only one of the two calmodulin-ubiquitin loci was represented in the library raising the possibility that the other is telomeric. An accurate physical map of a genome consisting largely of repetitive tandem arrays is dependent on obtaining long (>30Kbp) contiguous DNA fragments. Therefore the use of the YAC cloning system may greatly facilitate the generation of truly representative genomic libraries from trypanosomes.

C 438 THE 85 kDa TRYPOMASTIGOTE SURFACE ANTIGEN FAMILY OF *TRYPANOSOMA CRUZI*: HOMOLOGIES AND DOMAIN STRUCTURE. Garry B. Takle and George A.M. Cross, Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, NY10021.
The sequence of a cDNA (Tt34c1) encoding a *Trypanosoma cruzi* trypomastigote specific 85 kDa surface glycoprotein indicates that the peptide contains two eight amino acid motifs, SXDXGXTW, that are characteristic of bacterial neuraminidases and that occur in other glycosidases and sugar-attachment proteins. The deduced peptide sequence of the N-terminal domain of Tt34c1 is approximately 30% homologous to the complete sequences of neuraminidases from *Clostridium perfringens* and *C.sordellii*, and this domain may define the sugar-binding/glycosidase functional region of the protein. Tt34c1 is also highly homologous to other *T.cruzi* surface proteins, some of which contain extensive regions of C-terminal repeats, and alignment of available sequences indicates a highly conserved peptide motif (VTVXXVXLYNR) located just upstream of these repeats. The function of the C-terminal domain is unclear upstream of the hydrophobic C-terminus that is probably replaced by a glycosyl phosphatidylinositol membrane anchor, however, the polypeptide repeats may be used to extend the N-terminal functional domain from the surface of the membrane. Gp 85 is encoded by an extensive variant multigene family that is distributed throughout the genome, multiple copies of which are simultaneously transcribed, since gp85 clones, PCR amplified from trypomastigote mRNA, contained sequences varying at insertions and point mutations. Using expression constructs, derived from the N-terminal and C-terminal domains of Tt34c1, in bacterial and mammalian systems, the activities of the different regions of the glycoprotein have been investigated, and these data will be reported.

C 439 THE FUNCTION OF $CD4^+$ AND $CD8^+$ T CELLS IN EXPERIMENTAL Trypanosoma cruzi INFECTION, Rick L. Tarleton, T. Shields, M. Postan and M. Heiges, Department of Zoology, University of Georgia, Athens, GA 30602.
We have used antibody depletion protocols and mutant mice which fail to express the normal beta-2-microglobulin gene to determine the contribution of $CD4^+$ and $CD8^+$ to immunity to T. cruzi. Treatment of the normally resistant C57Bl/6 mouse with antibody to either $CD4^+$ or $CD8^+$ T cells resulted in high parasitemias and 100% mortality when the treatment was initiated at the time of infection. However if antibody treatment was delayed until day 15 of infection (for anti-CD4) or day 30 (for anti-CD8), only moderate increases in parasitemia and uniform survival of infection were observed. Further examination revealed that antibody treatment failed to completely deplete the targeted cell population in T. cruzi-infected mice eventhough the same treatment was effective in normal mice. Increasing the amount of antibody administered or combining thymectomy and antibody treatment likewise failed to achieve total T cell depletion in T. cruzi-infected mice during the acute phase of infection. Nevertheless, these antibody-treated T. cruzi-infected mice appeared to be *functionally* depleted based on their decreased resistance to infection and decreased antibody response to T. cruzi. T cell subset depletion in the post-acute and chronic stages of T. cruzi infection neither exacerbates the primary infection nor compromises resistance to re-infection. Mice in which the beta-2-microglobulin (beta-2-M) gene had been disrupted by homologous recombination fail to express class I MHC gene products and lack mature $CD8^+$ T cells. These mutant mice uniformly generate high parasitemias and die during the acute phase of T. cruzi infection. Despite their increased susceptibility to T. cruzi infection, beta-2-m -/- mice are more responsive in terms of lymphokine production, producing higher levels of both IL-2 and IFN-gamma in response to mitogenic stimulation. In addition, the beta-2-m -/- mice show essentially no inflammatory response in infected tissue. These results further support the conclusion that both $CD4^+$ and $CD8^+$ T cell subsets are required for initial control but are expendable after the first 30 days of T. cruzi infection in the mouse. In addition, these results support the role of $CD8^+$ T cells and/or class I expression in immunoregulation and pathogenesis in the infection.

C 440 *LEISHMANIA MAJOR*-SPECIFIC T CELL CLONES DERIVED FROM GENTICALLY-RESISTANT MICE, Richard G. Titus and Cynthia M. Theodos, Department of Tropical Public Health, Harvard School of Public Health, Boston, MA 02115
Leishmania major (*Lm*)-specific T cell clones were derived from the draining lymph nodes of *Lm*-infected, genetically-resistant C3H mice. FACS analysis revealed that the clones were $CD4^+$ T cells which expressed the $V\beta8.1$ chain of the $\alpha\beta$ T cell receptor. Adoptive transfer studies demonstrated that one clone, B3, exacerbated cutaneous leishmaniasis, while a second clone, E1, had a protective effect. Lymphokine analysis of these clones revealed that B3 produced interleukin 2 (IL-2) and interleukin 4 (IL-4) in response to *Lm*. In contrast, E1 produced IL-2, interferon gamma (IFN-γ), tumor necrosis factor (TNF) and migration inhibition factor (MIF). Since IFN-γ, TNF and MIF all play a protective role in cutaneous leishmaniasis, this suggests that protection against *Lm* involves several different cytokines. Studies investigating the fine antigenic specificity of these clones are revealing that B3 is specific for the gp63 surface glycoprotein of *Lm* and E1 is specific for a component of the lipophosphoglycan (LPG) surface molecule. Recent experiments suggest that E1 recognizes the contaminating proteins that co-purify with LPG and not the LPG molecule itself. Currently, we are attempting to isolate the protein that E1 recognizes within the LPG preparation. In addition, using a series of peptide fragments of gp63, we are determining the epitope of gp63 which is recognized by B3. Supported by NIH.

C 441 A SEQUENCE INSERTION TARGETING VECTOR FOR LEISHMANIA ENRIETTII, James F. Tobin and Dyann F. Wirth, Department of Tropical Public Health, Harvard School of Public Health, Boston, MA 02115
We have previously demonstrated that Leishmania enriettii contains the enzymatic machinery to mediate efficient interplasmidic homologous recombination. In this report we show that a sequence insertion targeting vector, pALT-Neo-Tub, can be inserted into the genome of L. enriettii by homologous recombination between α-tubulin sequences found in the plasmid and their homologs in the genome. pALT-Neo-Tub, a pBluescript derived vector containing the neo^r gene flanked by the α-tubulin intergenic and α-tubulin coding sequences, was used to transfect cells to G418 resistance. Analysis of the DNA from the drug resistant clones indicates that all of the insertion events are restricted to the α-tubulin gene repeats. As little as 200 base pairs of sequence homology between the plasmid and the genome is required for integration. Nonhomologous recombination events are not detected. These results indicate that exogenous DNA sequences can be integrated into the L. enriettii genome provided that the are flanked by homologous DNA sequences.

C 442 MOLECULAR RECOGNITION OF Trypanosoma cruzi TRYPOMASTIGOTES BY HOST CELLS, Fernando Villalta and Maria F. Lima. Division of Biomedical Sciences and Department of Microbiology, Meharry Medical College, Nashville, TN 37208.
We have purified a membrane 83 kDa glycoprotein from trypomastigote forms of T. cruzi which binds to host cells in a ligand receptor interaction manner. This purified T. cruzi adhesion molecule binds to host cells and blocks the binding and internalization of trypomastigotes into host cells in a concentration dependent manner. This cell adhesion molecule is developmentally regulated in the cell cycle of the parasite, since it is expressed in invasive trypomastigotes, but not in non-invasive epimastigotes. The expression of this adhesion molecule is up- and down-regulated in T. cruzi trypomastigote clones of different invasive abilities, indicating that the mechanisms involved in the regulation of the expression of this adhesion molecule in the trypomastigotes may modulate parasite virulence and infection in the mammalian host. N-glycanase treatment of this purified adhesion molecule releases complex-type oligosaccharides. We have produced a battery of monoclonal antibodies against the native trypomastigote cell adhesion molecule which recognize the 83 kDa on the parasite surface. One of these monoclonal antibodies (IgM) recognizes a cellular attachment domain in the trypomastigote cell adhesion molecule and inhibits the attachment and internalization of highly invasive trypomastigote clones into host cells in a concentration dependent manner. During the attachment of invasive trypomastigotes to host cells, trypomastigotes bind to surface polypeptides of molecular weight 45 and 50 kDa on myoblasts. These polypeptides may function as host cell receptors for the parasite. (Supported by NIH grant AI 25637, AID grant DAN 5053-G-00-0030-00 and NSF grant RII-9005826).

C 443 CHROMOSOMAL AND EXTRACHROMOSOMAL VARIATION IN T.CRUZI. Wilma Wagner and Magdalene So. Department of Microbiology and Immunology, Oregon Health Sciences University, Portland, OR 97201.
We examined genomic alterations that could reflect the broad spectrum of biological differences among T. cruzi strains. DNA mediated changes among four strains were indicated by variation in the size and number of chromosomes analyzed under pulsed-field conditions for the separation of linear DNA. When we modified the pulsed-field separation conditions for T. cruzi DNA we also identified a 75 kbp large extrachromosomal circular DNA (LED) that is stable during developmental conversion of T. cruzi. LED contains rRNA, spliced leader (sl) sequences and about 50 copies of a 196 bp repeat encoded on all chromosomes of the T. cruzi Y strain. DNA structures similar to LED exist in other Kinetoplastida that do not contain minichromosomes. The sequences encoding rRNA, the sl and the 196 bp repeats varied on LED from different T. cruzi stains and other Kinetoplastida. In T. cruzi Y trypomastigotes the amount of sl repeats in LED decreased over time. We previously observed changes in the chromosomal location of the sl clusters in epi- and trypomastigotes of the Y strain. Taken together, our data suggest that the sl clusters on the chromosomes as well as on LED undergo genomic rearrangements in T. cruzi.

C 444 Molecular Cloning and Sequencing of *Giardia lamblia* Virus Genomic cDNA. Alice L. Wang, Kathy A. Shen, and Ching C. Wang, Department of Pharmaceutical Chemistry, University of California, San Francsico, CA 94143-0446

Giardia lamblia virus (GLV) is a non-segmented dsRNA virus that belongs to the family of Totiviruses. It has been found in approximately one-third of the isolates of *G. lamblia* and does not infect other protozoa tested. During replication, it generates a 7 kb single-stranded RNA transcript (ssRNA) that is the message as well as the replicative form of its double-stranded genome. Using gel-purified ssRNA as template to synthesize the cDNA, several partial GLV-cDNA fragments have been cloned into the pBluescript vector. Viral origin of these inserts are verified by Southern and Northern hybridizations. S1 nuclease protection reactions are further carried out to eliminate possible cloning artifacts or sequence rearrangements. Positive clones are subsequently bridged by a combination of primer-extension and polymerase chain reaction (PCR) to generate large clones of overlapping regions. Using this strategy, we have obtained several larger clones and combined the nucleotide sequences into 2 large discrete regions, consisting of respectively 2,814 and 1,052 nucleotides (nt). Each of these regions shows one open-reading frame (ORF) throughout, of 938 and 350 amino acids (aa) respectively. The ORF of 938 aa contains 15% basic amino acids, has an estimated pI of 9.7 and shows all consensus motifs of RNA-dependent RNA polymerases (RDRP), whereas the other ORF of 350 aa has a predicted pI of 4.9 and shares limited homology with those of Kunjin virus polyprotein.

C 445 THE EFFECT OF PROTEIN SYNTHESIS INHIBITORS ON GP63 RNAS, M.E. Wilson, K.E. Paetz, and J.E. Donelson, Departments of Medicine and Biochemistry, Univ. of Iowa, Iowa City, IA 52242
The amount of the major surface glycoprotein gp63 expressed by *Leishmania donovani chagasi* (*Ldc*) promastigotes increases 10-fold as the parasites develop from log to stationary phase *in vitro*. This coincides with an increase in the virulence of the organism. We previously showed that RNAs transcribed from three different classes of gp63 genes are expressed differentially during development of *Ldc* promastigotes. 2.7 kb RNAs transcribed from Log class gp63 genes are expressed by promastigotes in log phase growth, whereas 3.0 kb RNAs transcribed from Sta class gp63 genes are expressed primarily by promastigotes in stationary phase growth. To begin investigating the regulation of these different gp63 gene transcripts, we incubated promastigotes in inhibitors of protein synthesis prior to extraction of RNA. Northern blots showed that the steady state level of Log class gp63 RNAs increased 11 to 16 fold after incubation in cycloheximide, whereas the Sta class gp63 RNAs increased only 2-fold. Tubulin RNAs increased 2-3-fold after incubation in cycloheximide. The increase in Log gp63 RNAs began 30 min. and was maximal 10.5 hrs. after the addition of cycloheximide. A similar superinduction of the Log gp63 RNA was caused by inhibitors that act at different steps in translation, including pactamycin which inhibits initiation, puromycin that causes premature termination, anisomycin that inhibits peptide bond formation, and emetine that inhibits translocation. Thus the effect was not likely due to specific degradation of Log RNAs during its translation. The increase in Log gp63 RNA was prevented by actinomycin D, a transcription inhibitor, suggesting the cycloheximide effect was observed only when the RNA was being actively synthesized. We measured the $T_{1/2}$ of Log gp63 RNAs in intact promastigotes after preincubation in buffer (control) or cycloheximide. The $T_{1/2}$ of the Log gp63 RNA was 30 min. in control promastigotes, and increased to 4 hrs. after incubation in cycloheximide. Cycloheximide did not prolong the $T_{1/2}$ of tubulin RNAs to a similar extent. Thus Log gp63 transcripts have a short $T_{1/2}$, which is maintained only during ongoing synthesis promastigote proteins. Highly labile negative regulatory proteins, such as RNases, may be responsible for specific degradation of Log gp63 RNAs.

C 446 THE INVOLVEMENT OF A B CELL MITOGEN IN THE DEVELOPMENT OF ARTHRITIS IN *BORRELIA BURGDORFERI* INFECTED MICE. Liming Yang, Barbara Araneo, Ying Ma, Robert Schoenfeld, and Janis Weis. Division of Cell Biology and Immunology, Department of Pathology, University of Utah School of Medicine.

Initial studies on the specific response in the mouse to the Lyme disease pathogen, *B. burgdorferi*, revealed that lymphocytes from naive animals proliferated vigorously when incubated with a sonicated antigen preparation. This has been further characterized as a B cell mitogen with the ability to stimulate proliferation and immunoglobulin production. The bacteria also possess potent IL-6 stimulatory activity causing splenocytes and endothelial cells to produce IL-6. In order to determine if the B cell mitogen plays a role in the development of arthritis we have utilized the murine model of Barthold, et al (J Inf Dis, 162:133). After intradermal injection of spirochetes into the C3H/He strain of mice 100% of animals will develop arthritis in the rear ankles, beginning at 10-14 days and peaking at 3-4 weeks. Histology indicates proliferating synovium and inflammation of tendons, with no breakdown of cartilage or bone. Concurrent with the joint swelling is elevation of serum IL-6 and a 10-fold increase in IgG. In contrast, the BALB/c mouse, which is becomes infected but does not develop severe arthritis, also does not display an elevation in IL-6 and has only a modest increase in IgG. Further studies of the kinetics of arthritis development in the C3H/He mouse have indicated that the arthritis is always associated with elevation of IL-6 and circulating IgG. Furthermore, the enlarged lymph nodes associated with *B. burgdorferi* infected animals primarily reflect expansion of the B cell population, with plasma cells observed in smears prepared from lymphoid tissues. The expansion of B lymphocytes and increase in total IgG levels are consistent with a B cell mitogen acting in concert with elevated IL-6. We hypothesize that self-reactive antibodies may be present in the population of expanded IgG and that these may play a role in the pathogenesis of arthritis. The host genes involved in the arthritis are not known, however, comparison of the C3H/He ($H\text{-}2^k$) mouse with the congenic C3H/SW ($H\text{-}2^b$) has indicated than the MHC haplotype does not determine the development of arthritis. This suggests that genes outside the MHC, perhaps affecting IL-6 levels, are involved.

Late Abstracts

IDENTIFICATION AND CHARACTERIZATION OF TRANS-SPLICED GENES IN SCHISTOSOMA MANSONI. Richard E. Davis, Sandy Koepf, Hardeep Singh, Cara Stahlman, and Lee Niswander, Department of Biology, San Francisco State University, 1600 Holloway Ave, San Francisco, CA 94132. We recently demonstrated that a subset of mRNAs in Schistosoma mansoni contain a 36-nucleotide spliced leader (SL) at their 5' termini (PNAS 87:8879, 1990). The schistosome spliced leader RNA (SL RNA) is unique, exhibiting no sequence identity with trypanosome or nematode SL RNAs, and contains only two, rather than three, computer-predicted stem loops. cDNA libraries enriched for schistosome mRNAs that contain spliced-leaders have been constructed and several mRNAs and their genes are currently being characterized. Two genes have been selected for in depth analysis as they are single copy genes which produce abundant trans-spliced mRNAs. Full length adult cDNAs corresponding to these mRNAs (one cDNA encodes a protein homologous to the glycolytic enzyme enolase) have been sequenced and primer extension sequencing of the mRNAs has confirmed that the spliced leader is terminally located. These mRNAs are also expressed and trans-spliced in cercaria. Sequence analysis of these genes indicates that both cis- and trans-splicing occur within the same transcripts. We previously have shown in HMG-CoA reductase that an internal exon can act as a splice acceptor for either cis- or trans-splicing. Analysis of sequences upstream from the acceptor site for trans-splicing indicates that there is considerable potential for base-pairing (8/8) between sequences immediately 3' of the SL RNA splice donor site and sequences 45 bases 5' of the trans-splice acceptor site in both HMG-CoA reductase and enolase genes that might be involved in mediating and facilitating interaction between the SL RNA and the primary transcript undergoing trans-splicing. Preliminary RNAase mapping and PCR analyses demonstrate RNA processing intermediates which suggest that cis-splicing of several 5' introns in the enolase gene precedes trans-splicing of the spliced leader to the trans-splice acceptor site. In situ hybridization analyses indicate that the expression of the schistosome spliced leader RNA likely occurs in all tissues, but that expression is highest in reproductive organs and in discrete foci of cells in the parenchyma. Expression of the trans-spliced enolase mRNA is highest in discrete foci of cells in the parenchyma, and unexpectedly, is not expressed highly in muscle or regions associated with the tegument. These studies may provide some insight into the function and biological significance of the spliced leader in the expression of these genes as well as the determinants, mechanism, and regulation of trans-splicing in schistosomes.

In contrast to nematodes, we have been unable to demonstrate primary sequence conservation of the schistosome leader in several other flatworms, including several other trematodes. We are currently working on developing assay to identify and clone spliced leader RNAs from other invertebrates, particular parasitic flatworms.

PROTECTION OF AOTUS MONKEYS FROM MALARIA INFECTION BY RECOMBINANT HYBRID PROTEINS, Erika Hundt, Bernhard Knapp, Burkhard Enders and Hans A. Küpper, Research Laboratories, Behringwerke AG, Marburg, F.R.G.

Based on investigations on the malarial blood stage antigens SERP, HRPII and MSAI from Plasmodium falciparum we have expressed two hybrid proteins in E. coli containing selected partial sequences of these antigens. Antibodies raised against both hybrid proteins in rabbits and Aotus monkeys recognize the corresponding P. falciparum polypeptides. In two independent trials using 13 animals we could show that immunization of Aotus monkeys with either of the two hybrid proteins administered in an oil-based well tolerated formulation can protect the animals from an experimental P. falciparum infection.

CHARACTERIZATION OF A *Trypanosoma cruzi* COMPLEMENT BINDING PROTEIN WITH FUNCTIONAL AND GENETIC SIMILARITIES TO THE HUMAN COMPLEMENT REGULATORY PROTEIN, DECAY ACCELERATING FACTOR,

Karen A. Norris*, Bonnie Bradt‡ and N. Cooper‡ , *University of Pittsburgh School of Medicine, Pittsburgh, PA 15261 and ‡ Research Institute of Scripps Clinic, La Jolla, CA 92034.

Evasion of destruction by the host complement (C) system is an essential event in the establishment of infection by many microorganisms. In the case of *Trypanosoma cruzi*, lysis of the bloodstream forms requires antigen-specific antibodies and proceeds mainly via the alternative complement pathway (AP). One target for such antibodies is a 160 kDa surface glycoprotein, which we have purified and shown to be a C regulatory protein. Biochemical and genetic analyses of gp160 reveal similarities to human decay accelerating factor (DAF). The *T. cruzi* gp160 protein restricts C activation by binding C3b and inhibiting AP C3 convertase formation and stability. The protein is anchored in the parasite membrane via a glycosyl phosphatidylinositol linkage, similar to DAF. Using anti-gp160 antibodies, we have isolated a portion of the gp160 gene and found that it shares significant DNA sequence homology with the human DAF gene. Anti-gp160 antibodies which mediated C lysis of the parasites were found to inhibit gp160-C3b binding. Antibodies to unrelated *T. cruzi* surface glycoproteins were not able to support C lysis or block C3b binding. Based on these results, we propose that the mechanism of antibody dependent, antigen specific lysis of *T. cruzi* via AP is the result of specific anti-gp160 antibodies which block the C regulatory activity of gp160 at the parasite surface. This allows unrestricted deposition and amplification of C on the parasite surface resulting in lysis.

DETECTION OF ORNITHINE DECARBOXYLASE ACTIVITY IN RAT *PNEUMOCYSTIS CARINII* LYSATES

Muhamed Sarić and Allen B. Clarkson, Jr., Department of Medical and Molecular Parasitology, New York University Medical Center, New York, N.Y. 10016

Although the specific ornithine decarboxylase (ODC) inhibitor α-difluoromethylornithine (DFMO) has been successfully used as an anti-*Pneumocystis carinii* agent in both animals and humans, the activity of the enzyme target has been reported to be absent from the parasite. We have reexamined *P. carinii* and found ODC to be present.

Using a novel isolation technique consisting of tissue homogenization, differential centrifugation and enzymatic digestion, we first separated *P. carinii* from the lungs of dexamethasone-treated Sprague-Dawley female rats. The parasite cells were then lysed and ODC activity measured upon the addition of carboxyl carbon-labeled ornithine as a substrate. The enzymatic assay initially revealed only minor, sporadic and non-reproducible ODC activity. However, after removing low-molecular weight compounds, significant and reproducible ODC activity was present and amounted to 15.7 pmol $^{14}CO_2$/mg of protein/hour. Moreover, the activity was linear with respect to protein concentration and was inhibitable by DFMO.

Despite the fact that our isolation procedure yielded unusually pure parasites, one could argue that the measured activity was due to the presence of the host ODC released from either lung or inflammatory cells. However, the finding that the activity in cytosolic extracts of normal rat lungs was only 15% of that in isolated parasites argues against contamination by the host enzyme. Furthermore, inflammatory response could not account for our results since the activity in cytosolic extracts of whole, *P. carinii*-infected rat lungs was only one-third of that in isolated parasites. In conclusion, our work indicates that *P. carinii* has an active polyamine biosynthetic pathway and this has important implications for the development of anti-*Pneumocystis* therapies based on polyamine biosynthesis inhibitors.

SYNTHETIC BIOTIN LABELING OF OLIGONUCLEOTIDES FOR USE AS SPECIES-SPECIFIC PROBES FOR THE DETECTION OF FILARIAL PARASITES.

Steven A Williams[2], Catherine Poole[1], David Landry[1], and Larry A. McReynolds[1]. [1]New England Biolabs, Beverly, MA 01915 and [2]Department of Biological Sciences, Smith College, Northampton, MA 01063.

We have developed a novel synthetic approach for the incorporation of biotin into a series of species-specific oligonucleotide probes for the detection of filarial parasites.The probes are designed to detect species-specific regions of a highly repeated DNA sequence found in all species of *Brugia*. The synthetic method described in this paper was used to construct oligomer probes tailed on the 5' end with 30 to 46 biotinylated uridine residues. Probes with 46 biotins were found to be more sensitive than probes with 30 biotins. We also found that alternating the biotinylated uridine residues with non-biotinylated thymidine residues improved the sensitivity of the probes. Melting temperature studies indicated that the long tails (up to 91 nucleotides) had only a minimal effect on the T_m of the probes. Conditions were found that optimized the sensitivity of the probes while maintaining their species-specificity. Using these conditions, individual parasites from blood were identified *in situ* in a species-specific fashion using a chemiluminescent detection system . This method of non-radioactively labeling oligonucleotides for the detection of infectious agents will enable the practical use of such probes in endemic regions in developing countries.

THE EFFECT OF PROTEIN SYNTHESIS INHIBITORS ON GP63 RNAS,

M.E. Wilson, K.E. Paetz, and J.E. Donelson, Departments of Medicine and Biochemistry, Univ. of Iowa, Iowa City, IA 52242

The amount of the major surface glycoprotein gp63 expressed by *Leishmania donovani chagasi* (*Ldc*) promastigotes increases 10-fold as the parasites develop from log to stationary phase *in vitro*. This coincides with an increase in the virulence of the organism. We previously showed that RNAs transcribed from three different classes of gp63 genes are expressed differentially during development of *Ldc* promastigotes. 2.7 kb RNAs transcribed from Log class gp63 genes are expressed by promastigotes in log phase growth, whereas 3.0 kb RNAs transcribed from Sta class gp63 genes are expressed primarily by promastigotes in stationary phase growth. To begin investigating the regulation of these different gp63 gene transcripts, we incubated promastigotes in inhibitors of protein synthesis prior to extraction of RNA. Northern blots showed that the steady state level of Log class gp63 RNAs increased 11 to 16 fold after incubation in cycloheximide, whereas the Sta class gp63 RNAs increased only 2-fold. Tubulin RNAs increased 2-3-fold after incubation in cycloheximide. The increase in Log gp63 RNAs began 30 min. and was maximal 10.5 hrs. after the addition of cycloheximide. A similar superinduction of the Log gp63 RNA was caused by inhibitors that act at different steps in translation, including pactamycin which inhibits initiation, puromycin that causes premature termination, anisomycin that inhibits peptide bond formation, and emetine that inhibits translocation. Thus the effect was not likely due to specific degradation of Log RNAs during its translation. The increase in Log gp63 RNA was prevented by actinomycin D, a transcription inhibitor, suggesting the cycloheximide effect was observed only when the RNA was being actively synthesized. We measured the $T_{1/2}$ of Log gp63 RNAs in intact promastigotes after preincubation in buffer (control) or cycloheximide. The $T_{1/2}$ of the Log gp63 RNA was 30 min. in control promastigotes, and increased to 4 hrs. after incubation in cycloheximide. Cycloheximide did not prolong the $T_{1/2}$ of tubulin RNAs to a similar extent. Thus Log gp63 transcripts have a short $T_{1/2}$, which is maintained only during ongoing synthesis promastigote proteins. Highly labile negative regulatory proteins, such as RNases, may be responsible for specific degradation of Log gp63 RNAs.

REGULATION OF GENE EXPRESSION FROM THE HSP83 CLUSTER OF LEISHMANIA MEXICANA AMAZONENSIS IN TRANSFECTION STUDIES

M. Shapira, M. Argaman, R. Ali and E. Pinelli. Department of Membrane Research and Biophysics, Weizmann Institute of Science, Rehovot 76100, Israel

Regulation of expression from the hsp83 gene cluster of the digenetic parasite Leishmania mexicana amazonensis was selected as a model for exploring temperature controlled gene expression. The first gene copy from this cluster along with 10 kb of flanking sequences were cloned, and an intergenic region was found upstream to the cluster. A plasmid in which the chloramphenicol acetyltransferase (CAT) reporter gene was cloned inbetween two similar intergenic regions derived from an internal repeat unit of the hsp83 cluster was electroporated into Leishmania parasites, resulting in CAT enzymatic activity which was increased upon heat shock (35°C). The presence of intergenic sequences both upstream and downstream to the reporter gene was necessary for expression, and SV40 eukaryotic termination sequences could not replace the leishmanial intergenic region cloned 3' to the CAT gene. S1 analysis of hsp83 mRNA showed that the major part of the intergenic sequences was transcribed, and mostly present as 3' non translated extensions. Deletion analysis of intergenic sequences cloned upstream to the CAT gene showed that removal of the original AG splice acceptor site did not eliminate CAT expression, nor did it affect the temperature inducibility of CAT expression. Deletion analysis of the intergenic sequences cloned downstream to the CAT reporter gene showed that these 3' extensions were essential mainly for maintaining increased expression at elevated temperatures. The data presented indicate that temperature induced gene regulation in Leishmania may function by mechanisms other than control of transcriptional initiation.