MOLECULAR STRATEGIES OF PARASITIC INVASION

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Keynote Address

C0 THE CACHEXIA ASSOCIATED WITH PARASITIC INFECTIONS: THE ROLE OF CACHECTIN/TNF, Anthony Cerami and Bruce Beutler, Laboratory of Medical Biochemistry, The Rockefeller University, 1230 York Avenue, New York, NY 10021

Animals or people infected with parasites frequently display a cachexia (from Greek "I have it bad") or body wasting. In the past this has been attributed to the diversion of energy supplies from the host to the parasite. However, some parasites e.g. trypanosomes, in small numbers can promote severe wasting.

For the past several years we have been studying the biochemical basis of this phenomenon. In brief, it has been found that macrophages, in response to a number of stimuli, (e.g. endotoxin, malaria, and trypanosomes) produce a 17Kd protein, cachectin, that has the ability to selectively switch cells and animals from an anabolic state to a catabolic state. During structural studies of cachectin it was observed that mouse cachectin had 80% homology with human tumor necrosis factor (TNF) and had TNF bioactivity. Mouse cachectin has since been cloned and sequenced; extensive homology (> 80%) between the two is observed. Of considerable interest is the observation that mouse cachectin and human TNF had a conserved sequence containing several tandem repeats of the octamer TTATTTAT in the 3' untranslated portion of the message. A similar sequence is also noted in this region for a number of other immune mediators e.g. lymphotoxin, the interferons, IL-1, GM-CSF, and fibronectin. Whether this sequence plays a role in the expression of these endotoxin-induced genes is an obvious question.

Once cachectin is produced by the macrophage it enters the bloodstream, and like other hormones, prompts a number of biological responses, many of which are still being catalogued. Specific high affinity receptors have been noted in a number of tissues, e.g. adipocytes, muscle cells, and liver. In the case of the adipocyte, the binding of cachectin to its receptors induces the cell to turn off synthesis of specific mRNAs that encode anabolic enzymes. As a result, the adipocyte is no longer able to convert glucose to fatty acids or take up exogenous fats, and begins to lose stored lipid. Continual addition of cachectin to these cells results in an "in vitro cachexia". The mechanism for the selective suppression of the anabolic genes is not known.

One of the most intriguing biological activities associated with cachectin, in addition to its role in cachexia, is its role in the induction of endotoxin shock. Continual administration of cachectin to mice causes loss of appetite, weight loss, shock, and death of the animal, while anti-cachectin antibodies can protect mice from the lethal effects of endotoxin administration.

Cachectin production presumably offers an advantage to mammals that are infected; however, the deleterious aspects of cachectin in parasitic infections are most noteworthy. Further studies should give additional insight into this fascinating protein.

Genome Structure and Plasticity — I

C1 TELOMERES, E.H. Blackburn, J. Forney, C.W. Greider, D. Larson and E.A. Spangler, Department of Molecular Biology, University of California, Berkeley, CA 94720

The essential component of a telomere appears to be a simple G+C-rich sequence at the end of the chromosomal DNA. We have shown that the telomeric repeated sequences of ciliates (1,2), yeast (3) and Trypanosoma brucei (4) all have the common formula 5'G_{1-8} $(T/A)_{1-4}$ 3' on the strand with 5' to 3' polarity toward the chromosome end. During log phase vegetative divisions of the ciliate Tetrahymena, all the telomeres of the somatic nucleus were found to grow by 5-10 bp/generation, increasing three-fold in overall size. Addition of telomeric repeat sequences accounted entirely for the growth. In order to understand the mechanism of both telomere growth and the de novo synthesis of telomeres which takes place during the differentiation of the somatic nucleus of Tetrahymena, we analysed extracts of Tetrahymena cells for enzymic activity that might be involved in these processes. We have identified a novel activity in these extracts which adds telomeric G_4T_2 repeats to the 3' end of a synthetic DNA oligonucleotide primer of appropriate telomeric sequence(5). Synthetic oligonucleotide primers consisting of the telomeric sequences of Tetrahymena, yeast or Trypanosoma all functioned as primers in the addition reactrion, which efficiently utilized micromolar concentrations of primer and dNTP substrates. We propose that the novel telomere terminal transferase activity is involved in telomere synthesis in vivo.

1. Katzen, A.L., Cann, G.M. and Blackburn, E.H. (1981). Cell 24, 313-320.
2. Blackburn, E.H. et al. (1985). In "Genome Rearrangement", UCLA Symposia on Molecular and Cellular Biology. New Series Vol. 20, pp 191-203.
3. Shampay, J., Szostak, J.W. and Blackburn, E.H. (1984). Nature 310, 154-157.
4. Challoner, P.B. and Blackburn, E.H. (1984). Cell 36, 447-457.
5. Greider, C.W. and Blackburn, E.H. (1985). Cell, in press.

C2 GENES AND CHROMOSOMES OF **PLASMODIUM FALCIPARUM**. David J. Kemp, Jennifer M. Favaloro, Hans-D. Stahl, Ross L. Coppel, Lynn M. Corcoran, Graham V. Brown and Robin F. Anders. The Walter and Eliza Hall Institute of Medical Research, Victoria, 3050, Australia.

To analyse relationships among the many gene products of **Plasmodium falciparum** that are natural immunogens in man, we constructed cDNA libraries expressing **P.falciparum** antigens in **Escherichia coli.** So far, clones representing at least 20 different genes have been isolated by screening the libraries with human antibodies. Remarkably, of 14 clones sequenced to date, 13 encode antigens containing repetitive sequences. These repeats range in length from 2 to 50 amino acids and the sequences are repeated up to ~100 times. The fidelity of repetition varies dramatically, with very different patterns and degrees of variation in different genes, both along the gene and between different isolates. Mutations in any one repeat are often found spread throughout the repeat array, an observation that can be readily explained by unequal crossing over. The technique of pulsed field gradient electrophoresis has enabled the resolution of at least 7 chromosomes from **P.falciparum.** The genes from repetitive antigens are distributed among these chromosomes and so there is no evidence for clustering of these genes in the **P.falciparum** genome.

C3 CHROMOSOME REARRANGEMENT EVENTS IN TRYPANOSOMA BRUCEI AND THEIR CORRELATION TO ANTIGENIC VARIATION, Lex H.T. Van der Ploeg, Cathy Shea, David Glass, Ramona Polvere and Gwo-Shu Mary Lee, Department of Human Genetics and Development, Columbia University, New York, NY.

The parasitic protozoan Trypanosoma brucei survives in the mammalian host by periodically changing the antigenic identity of its cell surface coat which consists of a single protein, the variant surface glycoprotein (VSG). This process, named antigenic variation relies on the differential expression of VSG genes. VSG gene activation can occur by different DNA recombinational mechanisms. As a result the VSG gene is always placed at one of several telomeric expression sites where it is transcribed. Transcription of expression sites is regulated, in a mutually exclusive manner. In order to understand regulation of transcription at expression sites we separated the chromosomes of T.brucei and determined the chromosomal location of expression sites in different variants (1,2). We found that chromosome rearrangements can lead to translocation of expression sites at VSG switching (2). Furthermore different rearrangements; insertion of a transcription terminator (3), loss of the VSG gene and reciprocal recombinations (unpublished results) can occur at expression site switches. Since one switch-specific DNA recombinational mechanism does not take place we assume that random mutations can inactivate and perhaps activate expression sites. Subsequent selection in an immunocompetent host, for parasites with a coat consisting of one VSG only may allow survival of parasites that transcribe one expression site only. Frequent chromosome rearrangements may be a feature shared among protozoans (4) and we are trying to identify its origin.

(1) Van der Ploeg, et al., 1984, Cell 37:77-84.
(2) Van der Ploeg, et al., 1984, Cell 39:213-221.
(3) Cornelissen, et al., 1985, Cell 41:825-832.
(4) Van der Ploeg, et al., 1985, Science, 229:658-661.

Antigen Structure

C4 CHARACTERISTICS OF MALARIA ANTIGENS WITH REPETITIVE STRUCTURES. Robin F. Anders, *Dennis B. Scanlon, **Pu-tao Shi, Hans-D. Stahl, Pauline E. Crewther, Graham V. Brown Ross L. Coppel and David J. Kemp, The Walter and Eliza Hall Institute of Medical Research and *The Ludwig Institute of Cancer Research, Victoria 3050, Australia and **The Shanghai Institute of Biochemistry, Shanghai, People's Republic of China.

The majority of malaria antigens that have been cloned contain short sequence repeats which encode antigenic epitopes that are naturally immunogenic. Some antigens contain a single block of tandem repeats whereas other antigens contain 2 or more blocks of related repeat sequences. The non-repeat flanking or intervening sequences in these antigens often have regions containing an unusual predominance of charged amino acids. The repeat units in different antigens vary in size, number and degree of degeneracy. The repeats in some antigens are remarkably conserved within the species whereas in other antigens the equivalent molecule in different strains of **P.falciparum** may contain very different repeat structures. For example, the S-antigen of isolate FC27 is an 11 amino acid sequence which repeats ~100 times whereas the equivalent antigen in isolate NF7 contains about 40 repeats of an unrelated 8 amino acid sequence. The serological diversity of **P.falciparum** S antigens reflects different repeat structures which may vary in unit size, sequence and number of repeats. Antigenic analyses have revealed a network of cross-reacting epitopes encoded by the repetitive structures of **P.falciparum** antigens. We propose that these unusual features of malaria antigens may interfere with the normal maturation of high affinity immune responses that occurs as a result of antigen selection of somatic mutations in lymphoid cells.

C5 STRUCTURE AND VARIATION WITHIN VARIANT SURFACE GLYCOPROTEINS OF TRYPANOSOMA BRUCEI, Mervyn J. Turner, Merck Sharp & Dohme Research Laboratories, P. O. Box 2000, Rahway, New Jersey 07065.

Antigenic variation within the African trypanosome is mediated by changes in the composition of the surface coat of these organisms. The surface coat is made up of a matrix of approximately 10^7 identical glycoprotein molecules, characteristic for each variant antigen type and, therefore, termed a variant surface glycoprotein (VSG). VSGs are glycoproteins containing 450-500 amino acids and 7-17% carbohydrate by weight. The molecules are remarkable both for the extreme diversity in the protein sequence and for the existence of an unusual mechanism for attachment and release of the molecules from the plasma membrane. VSGs can be separated into two domains through the action of trypsin or other proteolytic enzymes. The N-terminal domain, comprising approximately two-thirds of the molecule, is hypervariable in sequence, although a distribution of cysteines is relatively conserved. Different VSGs can be grouped into families according to homologies found within the C-terminal domain which also contains the membrane attachment site. This consists of a novel glycolipid covalently bound to the C-terminal amino acid and which contains dimyristoyl phosphatidylinositol. Activation of a phospholipase C removes the dimyristoyl glycerol and leads to the release of the VSG. The extent to which VSG structure is conserved in the face of extreme hypervariation within N-terminal domain sequences is of great interest. X-ray crystallographic evidence from one VSG hypervariable domain shows a remarkably compact structure containing a well defined bundle of alpha-helices.

Genome Structure and Plasticity — II

C6 EXPRESSION OF A TANDEM GENE CODING FOR A SEGMENTED MRNA IN T. CRUZI, Paul M. Lizardi Antonio Gonzalez, Terry J. Lerner, Maria Huecas, Beatriz Sosa-Pineda, and Nadia Nogueira, Laboratory of Cell Biology, The Rockefeller University, New York, NY 10021 & Department of Medical and Molecular Parasitology, New York University Medical Center School of Medicine, New York, NY 10010.

Using a cDNA for an abundant Trypanosoma cruzi mRNA as probe, we have cloned and sequenced a gene which is organized in at least 20 nearly perfect tandem repeats of 940 base pairs. The 5' end of the mRNA has been sequenced by primer extension and found to contain a 35 nucleotide mini-exon (or spiced leader) sequence that is ubiquitous in trypanosome mRNAs. This sequence, however, is not present in the tandem genomic repeats which encode the exon containing the major portion of the mRNA. Previous studies have shown that in T. cruzi, as in other trypanosomes, the 35-nucleotide sequence is encoded by a separate tandem gene family. One model to explain the formation of a segmented mRNA invokes priming of transcription by a small RNA which contains the leader sequence at its 5' end. However, northern blot analysis of total RNA from T. cruzi epimastigotes reveals a ladder of molecules larger than the mature mRNA, which appear to be faithful multimeric copies of the tandem gene. The discrete sizes of these RNAs correspond to those expected for partially processed precursors. Furthermore, the data suggests that a large multimeric precursor is being modified at each of every possible polyadenylation or splicing site. These observations lend credence to the possibility of an alternative model where segmented mRNAs are generated by inter-molecular splicing (1).

1. See Gonzalez, et. al., (1985) Nucleic Acids Res., 13: 5789-5804.

C7 MINICIRCLE SEQUENCE ORGANIZATION AND DNA REPLICATION MECHANISMS IN ISOLATED KINETOPLASTS OF Crithidia fasciculata, Dan S. Ray, Larry Birkenmeyer and Hiroyuki Sugisaki, Molecular Biology Institute and Department of Biology, University of California, Los Angeles, CA 90024

The DNA minicircles contained within the kinetoplast of the Cf-C1 strain of the trypanosomatid Crithidia fasciculata have been found to be nearly homogeneous in sequence. More than 90% of the minicircles exhibit a common pattern of restriction cleavage sites allowing the construction of a physical map of this population. Sequence analysis of multiple clones from this major class of minicircles shows nearly complete homogeneity at the nucleotide level. Two copies of the 14 base pair "universal minicircle sequence" have been localized to specific sites located 180° apart on the minicircle map.

Kinetoplasts isolated from this strain show an ATP-dependent incorporation of deoxyribonucleoside triphosphates. Replication products formed in such in vitro reactions are similar to those observed in vivo. Minicircles are replicated as free molecules and are subsequently reattached to the growing DNA network. A highly gapped minicircle species appears to be an early intermediate in the replication process. In addition, intermediates have been obtained in which specific discontinuities are localized in the regions containing the 14 base pair conserved sequence. Each region has two sites at which a discontinuity can occur, one on each strand and separated by approximately 100 base pairs.

Generation of Antigenic Diversity

C8 MOLECULAR BASIS FOR ANTIGENIC VARIATION IN A RELAPSING FEVER BORRELIA SP., Alan G. Barbour, Arthropod-borne Diseases Section, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT 59840

Borrelia hermsii, a tick-borne agent of relapsing fever, undergoes an extensive antigenic variation in its vertebrate host. Abundant, surface-exposed proteins with differing primary structures determine the serotype of each organism. The genes for two of the variable proteins were cloned into and expressed in Escherichia coli. Selected DNA sequences from the cloned genes were used as probes of total DNA from borreliae of different serotypes. Variable protein genes are found in two forms: silent copies and expression-linked copies. Both forms are located on linear plasmids of 20-30 kilobases. Change in serotype in a population of borreliae appears to be the consequence of a transposition of a gene copy occupying a silent site on one plasmid to a site that is on another plasmid and is adjacent to an expression sequence.

C9 VARIANT SURFACE GLYCOPROTEIN GENES OF T. EQUIPERDUM: HOW THEY ARE COMPOSED AND THE ROLE OF THE DUPLICATIVE TRANSPOSITION MECHANISM

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Activation of silent variant antigen genes (VAGs) of African trypanosomes involves at least two steps. First, copies of the silent genes must be inserted into telomeric expression sites (ESs) by a process of duplicative transposition. The expression-linked copies (ELCs) thus formed are then subject to in situ regulation. In T.equiperdum, the VAGs are expressed in a loosely defined order with VAG-1 always being the first gene expressed in a non-immune rabbit. We have analysed several early and late VAGs in an effort to understand why there are early and late genes and how the order of expression is determined. Contrary to what has been proposed for T.brucei, we find that early gene silent copies are not uniquely located at telomeres but are found internally in chromatin. Early genes all have competent silent copies which are duplicated and transposed into ESs in toto. On the other hand late gene ELCs are all hybrid with the 5' ends composed of parts of at least two silent genes and the 3' ends coming from a third gene, probably the last gene to occupy the ES. In two independent events creating similar composite late VAGs the same 3' end was used. Furthermore, the donor of the middle part of the genes was shown to be a pseudogene with no open reading frame beyond the point at which it recombined with the 3' end donor. These results suggest a model for the determination of expression of VAGs. They also indicate that late VAG ELCs have much in common with immunoglobulin genes in higher eukariotes; i.e. the 5' ends of the genes vary by gene conversion or recombination amongst related silent genes and a composite 5' end is added to the 3' end in the ES. This, in turn, suggests that the "cassette" mechanism evolved in unicellular organisms in order to allow gene rearrangments and the maintenance of a "germ line" in a single cell.

C10 THE EFFECT OF IMMUNITY ON THE INDUCTION OR SELECTION OF VARIANT ANTIGENS IN MALARIA, L.H. Miller, F.W. Klotz, and D.E. Hudson, Malaria Section, LPD, NIH, Bethesda, Maryland.

Malaria merozoites express cell surface molecules which may be target antigens for vaccine development. We studied one such merozoite surface antigen of a monkey malaria, Plasmodium knowlesi. Monoclonal antibodies against the 140 kDa protein block erythrocyte invasion in vitro. Immunization of rhesus monkeys with the 140 kDa antigen affords partial protection against an uncloned isolate in vivo. Parasites from these partially-protected monkeys express variants of the 140 kDa antigen.

To determine the capacity of a cloned parasite to generate variant 140 kDa antigens, monkeys immunized with the 140 kDa antigen were challenged with clones of P. knowlesi. Variant parasites arose from the cloned inoculum. Antibodies which block erythrocyte invasion of the cloned inoculum in vitro were ineffective against the variant parasites. These data indicate that variant parasites arose from a cloned inoculum. Immunoprecipitations from metabolically labled parasite antigens revealed that one variant expressed a new protein of 70 kDa while another variant expressed a new protein of 160 kDa. These new proteins were immunologically cross-reactive with the parental 140 kDa antigen.

We then determined whether 140 kDa antigen expression is stable during chronic P. knowlesi infection. P. knowlesi infection will become chronic in a nonimmune animal if the early infection is suppressed with antimalarial drugs. Parasites recovered from such chronic infections expressed the normal 140 kDa protein. Thus variants for the 140 kDa protein only appear from cloned parasites under the influence of specific anti-140 kDa immunity.

Functional Aspect of Parasite Antigens

C11 THE ROLE OF AMOEBAPORE IN CONTACT-MEDIATED CYTOLYSIS, Ian M. Rosenberg, Diana Bach, Leslie M. Loew and C. Gitler, Department of Membrane Research and Unit of Parasitology, Weizmann Institute of Science, Rehovot 76100, Israel.

Entamoeba histolytica kills cells by contact-mediated cytolysis. The mechanism underlying this process must be of rapid onset because target cells round up and show marked zeiosis already 15 min following contact with the amoebae. We have identified in virulent *E. histolytica,* a remarkable ion-channel forming protein, amoebapore, that is the presumptive causal agent of the amoeba-induced target cell killing (1,2). Amoebapore is a protein with a subunit molecular wt. of 15,000. Within the cell, it exists as a supramolecular aggregate containing some 40 to 80 ion channels together with other proteins of unknown function. This particle does not appear to be surrounded by a membrane. On addition to a planar lipid bilayer it leads to simultaneous opening of the component channels giving increases in conductance of 1.6 nS in 1M KCL at -10 mV. The channels remain open for periods of more than 30 sec. On disociation from the aggregate amoebapore is found to exist as a dimer in a water-soluble form. Two isoforms of this dimer exist with pIs of 6.8 and 5.3 at a ratio of 4 to 1. The two isoforms have been purified extensively and both are active in forming ion-channels. However, these channels show a unit conductance of 20-40 pS and remain open for <1 sec. Upon lateral aggregation opening becomes concerted to a greater degree. Aggregation seems to be highly cooperative so that no intermediates other than the tetramer are observed. Intracellular amoebapore is externalized in a particulate form that may be labelled with lactoperoxidase. This particulate effectively depolarizes cells. The overall process will be discussed.

1. Lynch EC, Rosenberg IM, Gitler C: An ion channel protein produced by *Entamoeba histolytica*. EMBO J.1:801, 1982.

2. Gitler C, Calef E, Rosenberg IM: Cytopathogenicity of *Entamoeba histolytica*. Phil. Trans. R. Soc. Lond. B 307:73, 1984.

C12 IMMUNOBIOLOGY OF MURINE CUTANEOUS LEISHMANIASIS, Richard M. Locksley, Michael D. Sadick. Frederick P. Heinzel, Matthew C. Keifer, Seattle Veterans Administration Medical Center and Department of Medicine, University of Washington, Seattle, WA 98185

The development of cell-mediated immunity during the course of Leishmania major infection in resistant (C57BL/6) and susceptible (BALB/c) mice was analyzed using in vitro bioassays. Cells isolated from the lymph nodes draining the infected footpads were examined for their ability to proliferate and to generate IFN-γ in response to mitogen or leishmania antigens at 1,3,5,7 and 9 weeks after infection. Both strains of mice demonstrated a suppression in their proliferative responses to mitogen and antigens during weeks 3-5. Although C57BL/6 mice recovered responsiveness to both mitogen and antigens by week 7 (a period correlating with healing), BALB/c never regained reactivity to leishmania antigens despite the return of mitogen proliferation to baseline values. Supernatants from in vitro leishmania-antigen-stimulated cells from C57BL/6 contained measurable IFN-γ by 3 weeks after infection that peaked by 5 weeks. BALB/c never displayed the capacity to generate IFN-γ in response to antigens throughout infection, although IFN-γ was readily generated by challenge with mitogen.

Sections of the draining lymph nodes were examined using in situ immunochemistry to compare the phenotypes of the responding cells during the evolution of infection. BALB/c mice had a significantly greater proportion of T cells expressing the L3T4 (helper/inducer) marker than did C57BL/6 mice. By 6 weeks after infection L3T4+ cells had increased to comprise over 50% of node cells in BALB/c; only 21% of cells from C57BL/6 mice expressed this marker. As assessed by FACS analysis of lymph node cell suspensions, the ratio of L3T4+/Lyt 2+ (suppressor/cytotoxic) cells was 2.4 times greater in BALB/c than in C57BL/6 mice. The number of amastigotes continued to increase over the course of infection in BALB/c but disappeared after 5 weeks in C57BL/6. Macrophages centrally located within granulomas were consistently Ia positive in C57BL/6 mice after three weeks of infection.

Prior irradiation of BALB/c mice or prior treatment with monoclonal antibody L3T4 allowed subsequently infected BALB/c mice to recover, and was associated with the capacity of cells isolated from these mice to generate IFN-γ. Conversely, prior immunization with discrete leishmania antigens accelerated the disease in BALB/c mice. Leishmania antigens may prime immunosuppression in BALB/c mice via a critical population of radiosensitive L3T4+ cells.

C13 COLLAGENASES AND VIRULENCE IN E. HISTOLYTICA, Marcos Rojkind and José Luis Rosales Encina, Department of Biochemistry, Centro de Investigación y de Estudios Avanzados del I.P.N., México D.F. 07000.
Entamoeba histolytica possesses a collagenolytic activity that can be demonstrated when axenic cultures of trophozoites are incubated on native collagen gels. The activity is manifested, only, when the amoebas establish direct contact with the substrate; thus suggesting, that the enzyme is either localized on the plasma membrane, or that the mechanism of activation of the enzyme is a receptor mediated process. Subcellular fractionation of the amoeba indicates that only a small fraction of the total collagenolytic activity is present in the plasma membrane fraction. Most of the enzymatic activity is localized in a lysosomal-rich fraction. However, when the trophozoites are first incubated with a collagen substrate and then fractionated, most of the collagenolytic activity is localized on the plasma membrane. These results suggest an active mechanism of migration of the enzyme from the lysosomes to the plasma membrane upon interaction of the trophozoite with collagen. This process, tentatively named "activation", is accompanied by the appearance of electron-dense particles beneath the plasma membrane. These particles are first surrounded by plasma membrane and then secreted onto the collagen gel. At the site of deposition of these particles there is active collagen degradation. It is therefore suggested, that the particles may contain the collagenolytic activity -in addition to many other lytic activities- that the trophozoite secretes when interacting with the host during tissue invasion. In order to determine the possible implications of the collagenolytic activity in tissue invasion, this activity was determined in five strains of amoebas known to have different degrees of virulence. We showed that the collagenolytic activity is directly proportional to the capacity of the trophozoites to produce liver abscesses when inoculated into the portal vein of newborn hamsters. From these results we concluded that the collagenase of E. histolytica plays an important role in tissue invasion. The interaction of the amoeba with collagen triggers the activation of the trophozoite, i.e., packing of collagenase and other enzymes into de electron-dense bodies", and release of these particles to destroy the host's cells and matrix. We propose that "activation" is a receptor-mediated process and that blocking the receptor with a specific antibody could impair the capacity of the amoeba to invade tissues. Supported with Grants from the Edna McConnell Clark, MacArthur and Rockefeller Foundations.

C14 IMMUNOBIOLOGIC PROPERTIES OF THE 72k GLYCOPROTEIN (GP-72) OF TRYPANOSOMA CRUZI, Alan Sher, Keith Joiner, L. Vaughn Kirchhoff and David Snary, Laboratories of Parasitic Diseases and Clinical Investigation, NIAID, Bethesda, MD 20892 and Wellcome Biotechnology, Beckenham, Kent, BR3 3BS, UK

GP-72 is a major surface glycoprotein of M_r 72 (x 10^{-3}) originally detected on T. cruzi epimastigotes (EPI) by means of a monoclonal antibody WIC 29.26. Our evidence indicates that this molecule serves several important functions in the interaction of the parasite with its vertebrate as well as invertebrate hosts and because of its polymorphic expression is useful in intraspecies classification. Thus, since WIC 29.26 specifically inhibits the transformation of EPI into trypomastigotes in vitro, GP-72 may function as a receptor involved in the control of infective stage differentiation in the reduviid vector. In addition, vaccination with GP-72 has been shown to result in a stage-specific partial protection effective against metacyclic (MT) but not blood stage trypomastigotes. Although approximately 50% of T. cruzi strains and clones fail to react with WIC 29.26, all isolates examined possess GP-72 as defined by immunoflourescence and immunoprecipitation reactions with polyvalent anti-GP-72 antibodies. Isolates expressing the WIC 29.26 epitope on their surface appear to have a different set of biologic properties than those which are surface negative. In recent work GP-72 has been defined as the major parasite acceptor for C3 during activation of the alternative complement pathway (ACP) by EPI. Thus, after reaction of surface iodinated EPI with C8 deficient human complement and subsequent affinity purification of the C3-membrane molecule complex, preferential reaction of C3 with GP-72 is observed. In contrast, when the same experiment is performed with MT, which fail to activate the ACP, only minimal reaction with GP-72 occurs. These data suggest that evasion of the ACP by MT might result from structural modifications undergone by GP-72 during differentiation which render the molecule incapable of serving as a C3 acceptor. Alternatively, other changes in the surface of the MT may alter the conformation of the C3-acceptor complex thereby preventing binding of Factor B and subsequent amplification of the ACP. We are studying membrane structural changes occurring during differentaition of EPI into MT in an attempt to distinguish between these two hypotheses. These developmental modifications in GP-72 and in the expression of other surface glycoproteins will be discussed.

Molecular Recognition Between Host and Parasite

C15 INVASION OF SIALIC ACID-DEFICIENT ERYTHROCYTES BY PLASMODIUM FALCIPARUM PARASITES: EVIDENCE FOR RECEPTOR HETEROGENEITY AND TWO RECEPTORS. T.J. Hadley, G.H. Mitchell, L.H. Miller, Walter Reed Army Institute of Research, Washington, DC; Guy's Hospital, London; NIH, Bethesda, Md.

Normal erythrocytes (RBCs) made deficient in sialic acid by treatment with neuraminidase, and variant RBCs which lack sialic acid and galactose on glycophorin A (Tn RBCs), are resistant to invasion by Plasmodium falciparum parasites. Different degrees of resistance have been reported from different laboratories. We cultured four strains of P. falciparum, including the Camp and Thai-2 strains, in Tn RBCs. Only the Thai-2 strain survived. We compared the Thai-2 strain cultured in Tn RBCs (Thai-Tn) and the Camp strain cultured in normal RBCs in terms of their abilities to invade neuraminidase-treated normal RBCs (Neur-RBCs) and Tn RBCs. Invasion rates of Camp parasites into Neur-RBCs were 1 to 7% of invasion rates into normal RBCs. Invasion rates of Thai-Tn parasites into Neur-RBCs were 25 to 54% of invasion rates into normal RBCs. Thus, although Thai-Tn parasites require sialic acid for optimal invasion, they invade sialic-acid deficient RBCs 8 to 25 times more efficiently than Camp parasites. Similar results were obtained with Tn RBCs. Invasion rates of Camp parasites into neuraminidase-treated pure Tn RBCs (Neur-Tn RBCs) were as low as invasion rates into untreated pure Tn RBCs (<5% of controls). Invasion rates of Thai Tn parasites into Neur-Tn RBCs were 35-50% higher than invasion rates into untreated pure Tn RBCs. This further indicates that there is a difference in receptors between Thai-Tn and Camp parasites. Invasion of trypsin-treated erythrocytes (Tryp-RBCs) was reduced by 90% for both Thai-Tn and Camp parasites. The finding that invasion of Thai-Tn parasites was reduced by 90% with Tryp-RBCs and only 50% with Neur-RBCs indicates that, in addition to sialic acid, there is another trypsin-sensitive binding site important for invasion. We conclude that P. falciparum merozoites may have two receptors, one which binds to a sialic acid-dependent site and another which binds to a sialic acid-independent, trypsin-sensitive site. Camp merozoites may require binding to both sites to achieve invasion, whereas Thai-Tn merozoites may bind more efficiently to the sialic acid-independent site and thereby achieve invasion in the absence of sialic acid.

C16 LEISHMANIA-MACROPHAGE INTERACTION: ROLE IN INFECTION AND HOST PROTECTION. E. Handman and G.F. Mitchell. The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia.

Studies on the molecular basis of the specific parasite-host recognition in leishmaniasis, have identified both parasite receptor and macrophage ligand responsible for the initiation of infection. A **Leishmania major** promastigote receptor for macrophages is a lipid-containing glycoconjugate which can be labeled biosynthetically with [^{35}S]-sulphate, [^{32}P]-phosphate, [^{3}H]-glucose or [^{3}H]-palmitic acid. A hydrophilic extracellular form of the molecule can be detected and is produced by cleavage of the membrane form by an endogenous phospholipase C. The macrophage ligand has been identified as the Mac-1 antigen or the C3bi complement receptor because monoclonal anti Mac-1 antibodies inhibit parasite attachment to macrophages. Parasite variants lacking the receptor are taken up by macrophages via a different mechanism and are rapidly killed and degraded. These parasites are not infective in mice or hamsters. The purified parasite receptor can protect genetically resistant or susceptible mice from infection with virulent **L.major** promastigotes. However, the hydrophilic, carbohydrate antigen derived from it is a suppressogenic molecule which exacerbates disease.

C17 CRISIS FORMS IN FALCIPARUM MALARIA James B. Jensen, Department of Microbiology and Public Health, Michigan State University, East Lansing, MI 48824

Intraerythrocytic retardation of malarial parasite development, associated with a pending immunologic "crisis", was first described in Plasmodium brasilianum infections in Cebus monkeys by Taliaferro and Taliaferro in 1944, and is today routinely seen in rodent and simian infections. Several investigators have shown that when cell-mediated immune responses of rabbits, or mice are hyperstimulated by BCG infections and subsequent injections of endotoxin, their sera typically contains numerous cytokines, and induces typical crisis-form parasites in vivo and in vitro. One of the better characterized cytokines of BCG-LPS serum, Tumor Necrosis Factor (TNF), has been proposed as the cause of crisis forms. Jensen et al. were the first to demonstrate that human serum from malaria-immune persons living in Sudan caused marked retardation of development in cultured P. falciparum, producing typical crisis form parasites. Serum titers of this nonantibody crisis-form factor (CFF) correlated with endemicity of malaria, increased more than 300% during the malaria-transmission season, and were strongly associated with clinical immunity to malaria in Sudan. Despite these findings, the role of CFF in aquired immunity to malaria is still not clear. The activity of CFF is not influenced by the action of antioxidants, thus, it is not a free radical generator. The fact that crisis forms can be induced with BCG-LPS, cytokine-rich serum strongely implies that CFF may be a mononuclear cell-secretion product. Recent studies have shown that human recombinant, DNA-produced TNF was not active against P. falciparum, nor were any of the Interferons. Furthermore, since both TNF and Lymphotoxin are cytotoxic to ML929 cells, and human CFF is not, this antiparasitic factor is neither of these cytokines. Serum titers of CFF are consistantly higher in sera from adults than children, although children with sickle-cell trait, G-6PD deficiency and B-thalassemia have significantly higher concentrations than children without these polymorphisms. The antiparasitic activity is found in a 45-60kDa by column chromatography, thus by mol. wt. estimates, it is in the size range of a typical cytokine.

C18 **Induction of lectin activity in protozoa.** **H. Ward and M.E.A. Pereira. Tufts-New England Medical Center, Boston, MA 02111**

A unique lectin activity has been found in Giardia lamblia, Trypanosoma cruzi, Leishmania mexicana amazonesis, Toxoplasma gondii and Plasmodium falciparum. All these protozoa displayed a lectin activity induced by limited trypsin proteolysis that reacted most specifically with mannose-6-phosphate and O-phosphonomannanes. The activated lectin did not agglutinate erythrocytes treated with alkaline phosphatase. In all cases, the lectins was pelleted by centrifugation at 100,000 x g, it always emerged in the void volume of agarose columns, and it was readily soluble and stable in detergents. Lectin activity was produced by trypsinization of either intact or lysed parasites, although lectin titers were much higher in lysed cells. These findings indicate that a mannose-6-phosphate binding lectin can be produced in protozoan parasites by limited proteolysis and that the lectin is membrane-bound, partly in the plasma membrane.

Developmental Changes in the Parasite Life Cycle

C19 MOLECULAR GENETICS OF TOXOPLASMA GONDII, John C. Boothroyd(1), J. Lawrence Burg(1), Susana Nagel(1), Pilar Ossorio(1), Dalia Perelman(1), Lloyd H. Kasper(2), Patricia L. Ware(2), Jeff Prince (3), Somesh Sharma(3) and Jack Remington(3). (1)Department of Medical Microbiology, Stanford University School of Medicine, Stanford CA 94305; (2) Department of Medicine (Neurology), Dartmouth Medical School, Hanover NH 03756; (3) Department of Immunology and Infectious Diseases, Research Institute, Palo Alto Medical Foundation, Palo Alto CA 94301.

The protozoan parasite, Toxoplasma gondii, is a member of the class Sporozoa and order Coccidia. It is an obligate intracellular parasite which reproduces in a variety of cell types within its mammalian hosts. In humans, the most significant disease caused by the organism is in the developing foetus and immunosuppressed individuals. The well defined sexual cycle occurs only within the intestinal epithelium of felines, its definitive host. Using the techniques of recombinant DNA and monoclonal antibodies we have embarked on a series of investigations aimed at understanding several of the basic biological and disease-causing properties of the parasite. Specifically, we have used monoclonal and polyclonal antibodies against the major surface antigens of the parasite to isolate and characterize their respective genes. Partial or complete nucleotide sequences of some of these genes have been generated. We have also cloned and partially sequenced the unique alpha- and beta-tubulin genes of T. gondii. These latter data indicate that intervening sequences within the genes of Toxoplasma gondii are much more common than has been seen in the other parasitic protozoa; they also help to establish the phylogeny of the organism. We are currently asking whether the process of discontinuous transcription observed within the Kinetoplastida is operating in the synthesis of these genes. We are planning to take advantage of the well characterized sexual cycle and the recently developed procedures for chromosome separation (pulsed field gradient electrophoreseis) to investigate recombination within these protozoa.

C20 MOLECULAR MARKERS OF LEISHMANIA DIFFERENTIATION, Diane McMahon-Pratt, Richard Ismach, Charles Jaffe, Lesley Kahl, Pamela Langer and Alfred Pan, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06510

Molecules unique to each end stage of Leishmania differentiation are most likely to be crucial to the adaptive survival of the parasite in each of its "host" environments. Molecules specific for each developmental stage of Leishmania (promastigote/amastigote) have been identified by means of monoclonal antibodies. Promastigote stage specific proteins have been localized both by indirect immunofluorescence and electron microscopy to either the external membrane or the flagella of the parasite. Protein molecules of apparent molecular weights 68,000, 70,000 and 14,000 daltons were uniquely associated with the flagella of the promastigote. Protein molecules of apparent molecular weights of 24,000, 17,500 and 46,000 daltons were found to be associated with promastigote membrane. The 46,000 daltons (M-2) glycoprotein has been isolated and purified. Biochemical and structural analysis of the M-2 glycoprotein indicated that the molecule is relatively stable to proteolytic digestion. The stability of approximately half of the molecular mass of the M-2 protein was dependent upon disulphide bonds while the remainder of the molecule (a glycopeptide of Mr 22,000 to 27,000) was apparently resistant to proteolysis. This resistant glycopeptide contained all the carbohydrate and appeared to be the dominant immunogenic portion of the M-2 molecule. Data obtained using the radioiodinated hydrophobic probe 3- (trifluoromethyl)-3-(m-iodophenyl) diazirene (TID) indicated that M-2 is an integral membrane component and that the (Mr 27,000/22,000) glycopeptide contained sequences within the lipid bilayer of the external promastigote membrane. Pulse-chase and endoglycosidase H experiments indicated that the M-2 glycoprotein contains a single carbohydrate chain.

A number of molecules specific for the amastigote stage of Leishmania have also been identified. The molecular characterization of these molecules and a quantitative comparison between amastigotes obtaoned from infected tissue, cultured macrophage cell lines and axenic culture will be presented.

C21 PROTEOLYTIC ENZYME ACTIVITY DURING THE SCHISTOSOME LIFE CYCLE, George R. Newport, University of California, Berkeley, CA 94720.

The humoral immune response to Schistosoma mansoni (as judged by immunoprecipitation of in vitro translation products of the organism's RNA with serum from experimentally-infected mice and rats, or from naturally-infected human donors) was found to be directed at only a minor number (5-40) of proteins. Examination of the temporal expression of these immunogens revealed that the vast majority (90%, depending on source of antiserum) are synthesized both during the mammalian and molluscan stages of the schistosome life cycle. Two notable exceptions to this trend were noted (via biochemical and/or Northern analyses) to be highly-expressed proteolytic enzymes: (1) a serine protease synthesized while within the snail and fundamental to penetration of warm-blooded hosts and (2) a thiol dependent protease which plays a central role in digestion of hemoglobin ingested during the intramammalian phase of the life cycle. At the time of this writing, a 23 bp oligomer based on and specific to the NH_3 terminal sequence of the cercarial protease has been synthesized, and immunogenic portions of the adult worm "hemoglobinase" have been cloned and expressed in Escherichia coli.

C22 MITOCHONDRIAL GENE EXPRESSION DURING DEVELOPMENT IN TRYPANOSOMA BRUCEI, Kenneth Stuart, Jean E. Feagin, and Douglas P. Jasmer, Issaquah Health Research Institute, Issaquah, WA 98027

T. brucei employs glycolysis or oxidative phosphorylation for energy production in the bloodstream and procyclic (insect) stages of the life cycle, respectively. It contains a single large mitochondrion within which is a single DNA network composed of thousands of heterogeneous 1 kb minicircles and tens of homogeneous 22 kb maxicircles. We have sequenced four minicircles. They do not appear to have a protein coding function and are not transcribed but they conserve a general organization and contain insertion sequence-like elements. We have sequenced much of the maxicircle and have identified genes by comparison to metazoan mitochondrial DNAs. Except for the variable region, the entire maxicircle is transcribed on one strand or the other. Larger low abundance transcripts suggest that each maxicircle gene does not contain its own promoter but that maxicircle transcripts undergo processing. Intergenic regions exhibit a strong G versus C strand bias and the C rich strand is preferentially transcribed into relatively small RNAs. Surprisingly, two major polyadenylated transcripts were found for each protein coding gene but one. Each maxicircle protein coding gene is transcribed during all life cycle stages examined but specific maxicircle transcripts are differentially expressed and the abundance patterns vary independently for the various genes. Apocytochrome b and cytochrome c oxidase subunits I and II transcripts are low in abundance or absent in slender bloodstream forms and progressively increase in abundance in stumpy bloodstream forms and procyclic forms while the NADH dehydrogenase subunit 5 transcript shows the reverse pattern. A smaller fraction of the maxicircle transcripts are polyadenylated in bloodstream forms compared to procyclic forms. These studies suggest that regulation of the production of the mitochondrial respiratory system involves differential expression of mitochondrial genes in T. brucei.

RNA Processing and Gene Expression

C23 RNA PROCESSING AND CATALYTIC RNA, Sidney Altman, Madeline Baer, Heidi Gold, Cecilia Guerrier-Takada, Nathan Lawrence, George McCorkle and Augustin Vioque, Department of Biology, Yale University, New Haven, CT 06520

Several RNAs perform in vitro catalytic cleavage or self-cleavage and ligation reactions relevant to biologically important RNA processing events. Among these are the RNA subunit of E. coli RNase P, M1RNA, the precursor to Tetrahymena thermophila rRNA, and plant viroid RNA. In the former two cases the reactions in vitro are also known to reflect intra-cellular events.

The structure and mechanism of action of M1 RNA will be discussed in some detail. Both comparison of nucleotide sequences from different organisms and enzymological studies in vitro yield information regarding the secondary structure in solution of M1 RNA. Site specific mutagenesis and manipulation of the cloned gene for M1 RNA in in vitro transcription experiments have led to a greater understanding of the catalytic properties of the RNA. One third of the 3' terminal portion of MI RNA is not essential for catalytic activity provided that the 5' terminus is intact.

New approaches to the purification of the protein cofactor of E. coli RNase P and both subunits of the analogous enzyme extracted from HeLa cell nuclei involve affinity column chromatography.

C24 IDENTIFICATION AND ANALYSIS OF THE GLYCOLYTIC GENES OF TRYPANOSOMA BRUCEI.

F.R. Opperdoes*, P. Michels*, K. Osinga, W. Gibson, B. Swinkels and P. Borst, *International Institute of Cellular and Molecular Pathology, Brussels, Belgium and The Netherlands Cancer Institute, Amsterdam, The Netherlands.

We have identified and studied several of the genes coding for the glycolytic enzymes of T. brucei. An analysis of the cDNA and the genomic clones has not revealed any intervening sequences. The trypanosome contains one gene for triose-P isomearse and two tandemly linked genes for each aldolase and glyceraldehyde-P dehydrogenase (GAPDH). For P-glycerate kinase (PGK) three tandemly linked genes were found; one encoding the cytosolic isoenzyme, one the glycosomal isoenzyme and a gene coding for an as yet unidentified PGK-related protein with a 100 amino-acid insertion. Both GAPDH and both aldolase genes had identical codogenic sequences, whereas the two true PGK genes differed by only 5 %. In addition the glycosomal isoenzyme contained a C-terminal extension of 20 amino acids. All glycolytic genes analyzed revealed a sequence homology at the amino-acid level between 45 and 55 % with the corresponding proteins from other organisms. A striking characteristic of all glycosomal enzymes studied thus far is that they have a high isoelectric point as the result of substitutions and/or insertions, which have led to an excess of positively charged amino acids in the proteins. We assume that these positive charges either play an important role in import of the newly synthesized proteins into the glycosome, or in the proper functioning of the enzyme in the glycosomal matrix.

C25 DOUBLE-STRANDED RNA OF TRICHOMONAS AND GIARDIA. C. C. Wang and Alice L. Wang, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143

Trichomonas vaginalis and Giardia lamblia are facultative anaerobic protozoa classified as two of the most prevalent human pathogens in the world. The former parasitizes the vaginal tract effecting chronic vaginitis, whereas the latter resides in the intestine and causes severe diarrhea and dehydration. In our recent effort to isolate DNA from the two parasites cultivated axenically in vitro, we noticed the presence of a well-defined 5.5 kb, ethidium bromide-stainable band in T. vaginalis nucleic sample, and a 7.0 kb band in G. lamblia nucleic acid preparation. These two bands disappeared from agarose gel upon soaking the gel in alkali or digestion with pancreatic ribonuclease A. Tjey are readily digested by ribonuclease T1 in low salt buffer but become quite resistant to the enzyme in high salt (0.1 M NaCl). They are insusceptible to bleomycin A_2 and cannot be labeled by nick translation using DNase I and DNA polymerase I. The T. vaginalis 5.5 kb band has relatively high T_m of 82°C in 0.5X SSC with 7-15% hyperchromicity. Under electron microscope, both 5.5 kb and 7.0 kb bands appear as uniform-lengthed, linear double-strands. Denaturation in 30% DMSO leads to the appearance of single strands of the original lengths, suggesting that both 5.5 kb and 7.0 kb bands are linear, open-ended and double-stranded molecules. Of the 33 different strains or isolates of T. vaginalis examined, all contained the 5.5 kb double-stranded RNA (dsRNA) except the two metronidazole-resistant strains IR78 and CDC85, which had no detectable dsRNA. No similar dsRNA can be detected in Tritrichomonas foetus, Entamoeba histolytica or Trypanosoma brucei. More recent studies showed that the two dsRNA's can survive in crude homogenates treated with ribonuclease T1 and proteinase K, and sediment at 100,000 x g. Examination of the sediments under electron microscope revealed the presence of large numbers of spherical particles approximately 40 nm in diameter in both T. vaginalis and G. lamblia preparations. These are likely viral particles (named respectively TVV and GLV), and the two dsRNA's could be viral RNA's. TVV-like particles are not found in the metronidazole-resistant T. vaginalis. Preliminary tests showed that TVV could not transfect the drug-resistant strains to dsRNA-bearing cells. Nevertheless, as far as we can tell, this is the first time viruses have been positively identified in protozoa. The biological functions of the two viruses and the possible interaction between the dsRNA and the host genome will be investigated.

Molecular Genetics

C26 THE CS PROTEIN GENES OF PRIMATE MALARIAS: David Arnot, John Barnwell, Vincenzo Enea and Mary Galinski, New York University, New York NY 10016.

The genes encoding the circumsporozoite (CS) proteins of the human malaria Plasmodium vivax and the simian malaria P. cynomolgi (Gombak) have been cloned and completely sequenced. Comparison of these sequences with the published sequences of the CS genes of P. knowlesi and P. falciparum allows some indication of their relationships. The N- and C-terminal domains of the CS protein in P. vivax are closely related to the corresponding regions of the CS genes of the simian malarias, P. knowlesi and P. cynomolgi. In contrast, the homology between the CS genes of P. vivax and P. falciparum is very limited. The central domain of repetitive epitopes present in all CS genes is not conserved between any of the genes so far studied although marked constraints on the amino acid composition of the repeated amino acid sequences occur.

C27 SPECIFIC DNA PROBE FOR THE DIAGNOSIS OF P. FALCIPARUM MALARIA. R.H. Barker jr. and D.F. Wirth. Harvard School of Public Health, Boston, MA.

A genomic DNA library of Plasmodium falciparum was screened to detect clones containing DNA sequences which are highly repeated within the parasite genome. Several such clones were further analyzed to identify those which hybridize specifically with P. falciparum DNA, but not with DNA from Humans, P. vivax, or P. cynomolgi. One clone pPFT4 was found to recognize only P. falciparum DNA and its sequence is repeated greater than 10,000 times within the genome. This clone has been sequenced and shown to consist of 21 bp tandem repeats. A simple procedure was developed for spotting blood from infected patients directly after lysis onto nitrocellulose paper and identifying the malaria species on the basis of hybridization of parasite DNA with a species-specific probe. This technique appears to be sensitive enough to detect one parasite per 5 microliters of blood. Field validation of this probe is currently in progress in Thailand.

C28 TUBULIN GENES OF TRYPANOSOMA MEGA, Vivian Bellofatto, Peter Hevezi, George A.M. Cross, The Rockefeller University, New York, NY 10021

Trypanosoma mega is a protozoan parasite of toads that can be grown as single cell-derived colonies on agar plates containing rich or defined media. Genomic α and β tubulin clones were derived from a partial BamH1 T. mega library by hybridization with α or β tubulin sequences from T. brucei. The α and β tubulin genes exist as independent tandem repeats, 4.2KB and 2.7KB long, respectively. Each repeat is present approximately 10 times per haploid genome. Nuclease S1 mapping results identify the boundaries of the mature α tubulin transcript, direction of transcription, and indicate a distance of 1.9KB between the transcripts. Primer extension and sequencing results demonstrate that the 5' end of α tubulin mRNA bears a 35 base sequence, 85% homologous to the T. brucei mini-exon, which is absent in the corresponding genomic coding region. We are extending our understanding of transcription of tandemly reiterated genes by characterizing the flanking α tubulin sequences.

C29 MOLECULAR STUDIES OF GERM LINE GENES IN ASCARIS LUMBRICOIDES, Karen L. Bennett and Sam Ward. Carnegie Institution of Washington, Baltimore, MD. 21210.

Ascaris lumbricoides is a parasitic nematode of pigs and humans. During early embryogenesis Ascaris eggs undergo chromatin diminution. Between the 3rd and 5th cleavages 30% of the DNA is eliminated from all somatic precursor cells whereas the germ line precursors retain the full DNA complement. Others have shown that most of the eliminated DNA is satellite sequences. We have asked if a germ line-specific gene is also lost or altered during somatic cell chromatin diminution. Previously this laboratory has cloned genes which encode the major sperm proteins (MSP) of the nematode Caenorhabditis elegans and shown that the expression of the genes in this multigene family is testis-specific. Using a C.elegans MSP cDNA probe we have isolated the corresponding gene from an Ascaris cDNA library. In contrast to the large gene family of over 50 members in C.elegans, the MSP gene is present in only one or possibly 2 copies in Ascaris, although the protein is still the most abundant protein in sperm. Preliminary sequence analysis shows the two proteins are about 70% homologous. By Northern blot analysis, the MSP RNA is found only in the testis and not in the ovary or intestine, so the gene is germ line-specific. Comparison by Southern blot analysis of germ line and somatic DNAs (which have undergone chromatin diminution) shows no difference in MSP gene number or arrangement. Thus at least one germ line-specific gene is neither lost nor rearranged during chromatin diminution. We have also cloned actin and alpha-tubulin genes, which are predominantly expressed in somatic cells. Like the MSP gene, neither of these multigene families show any changes in DNA arrangement during chromatin diminution.

C30 AMPLIFICATION OF THE DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHETASE GENE OF LEISHMANIA MAJOR Stephen M. Beverley, Jeff Kapler, John Cordingley, Dept. of Pharmacology, Harvard Medical School, Boston,MA. Methotrexate-resistant Leishmania major (the R1000 line of the LT 252 strain) have been selected which bear two separate amplified DNAs in the form of extra-chromosomal circular DNAs (Beverley et al, Cell 38: 431, 1984). One of these extra-chromosomal DNAs, termed the R region, was proposed to contain the structural gene for the target of methotrexate, the bifunctional dihydrofolate reductase-thymidylate synthetase characteristic of all protists. Over-production of this enzyme was invariably associated with R amplification (loc cit., Coderre et al, PNAS 80: 2132 1983). We have now demonstrated by hybrid selection of mRNA as well as DNA sequencing that the R region does in fact contain the coding region for the DHFR-TS of Leishmania major. In addition, the R region encodes at least three other poly-A+ transcripts, which currently appear to be functionally unrelated genes co-amplified with the selected DHFR-TS gene. The levels of all four mRNAs are dramatically elevated within the R1000 line, relative to that observed in the parental wild-type line. No alterations in mRNA structure or relative expression of the four mRNAs have occurred as a result of amplification. In total these four mRNAs are encoded by approximately 25 kB of the 30 kB R region, suggesting an apparently high density of transcripts in the genome.

C31 Messenger RNA processing in Trypanosoma cruzi; addition of a 36 base leader element. Gregory A. Buck, John Swindle and Harvey Eisen. Medical College of Virginia, Richmond, Va. 23298; and Institut Pasteur, Paris, France 75015.

Most or all trypanosome mRNA bears a common 35-36 base leader element (LE). This LE is encoded in genomic sequences that need not be linked to the genes encoding the bodies of the mRNAs. Two models have been proposed for the mechanism of LE addition to trypanosome mRNA: 1) a transcriptional priming mechanism whereby the LE is required for transcription priming; and 2) an intermolecular splicing mechanism whereby the LE is added in a bimolecular splicing reaction. We have isolated and sequenced several T. cruzi cDNA clones which bear the LE. The T. cruzi LE is 36 nucleotides long and differs from the LE of the African trypanosomes at three contiguous sites. We are currently using these cDNA clones to analyze ^{32}P-labelled nascent transcripts from T. cruzi nuclei to determine which of the models; i.e. priming or intermolecular splicing, is correct. Our initial results suggest that nascent transcripts do not bear the LE, thus favoring the intermolecular splicing mechanism.

C32 PROCESSING OF RIBOSOMAL RNA IN *T. BRUCEI*. David A. Campbell, Ken M. Kubo and John C. Boothroyd, Stanford University, Stanford, CA 94305.

The processing of eukaryotic ribosomal RNA precursors typically involves the cleavage of a 5.8S species from sequences located between the mature 18S and 28S species. Additionally, trypanosomes cleave the 28S species generating two large species (28Sα and 28Sβ) and several smaller species ranging from 214 to 76 nucleotides in length. By comparison of the nucleotide sequence and S_1-nuclease-protected fragments, we have mapped the processing sites involved in the generation of 5.8S RNA. We show also that the cleavage producing 28Sα and 28Sβ generates the 214 nucleotide species (sRNA1).

C33 POSSIBLE DIFFERENTIAL CONTROL OF HEAT INDUCED PROTEINS IN Trypanosoma cruzi. Elizeu F. de Carvalho, José Francisco Carvalho, E. Rondinelli, R. Silva, C. Soares and F.T. de Castro. Instituto de Biofísica, UFRJ, R.J., Brasil

Data from our laboratory have shown that changes in the gene expression in T. cruzi can be induced by heat. As a response to the elevate temperature (37ºC) epimastigote, trypomastigote and amastigote cells increase the translation of at least 4 distinct proteins (103, 92, 75 and 61 KDa). However in the presence of actinomycin D (AMD) only the proteins of 92 and 75 KDa were increased. Pulse labelling experiments in the presence of AMD revealed that: 1) after 6h, at 29ºC, the 92 and 75 KDa proteins were not detected although they could still be induced by hyperthermia. On the other hand the 61 KDa protein was always present but not induced by heat; 2) after 6h, at 37ºC, the incorporation of the protein precursors was inhibited in the 92 and 75 KDa proteins but not in the 61 KDa protein. In these conditions the total amount of RNA either in cells at 29ºC or 37ºC in the presence or absence of AMD was the same. However, the poly A^+ RNA in the organisms that were treated with AMD at 37ºC fells to 50% of the control. The data above suggest that the response of T. cruzi to the heat treatment is independent of the mRNA synthesis for the 92 and 75 KDa proteins while the induction of the 61 KDa protein seems to operate at the transcriptional level.

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C34 DEVELOPMENTALLY REGULATED GENES IN TRYPANOSOMA BRUCEI

Christine E. Clayton, S. Vijayasarathy, Elliot J. Davidowitz, Patricia Wright and Michael R. Mowatt. The Rockefeller University, 1230 York Ave., New York, N.Y. 10021, USA.

Low stringency hybridization with a rabbit aldolase cDNA was used to select cDNA clones encoding fructose biphosphate aldolase in Trypanosoma brucei. A clone which is almost full length encodes a protein of 41,027 daltons which has 50% identity with rabbit aldolase A and slightly lower homology with B-type aldolases. The homologous mRNA is at least six-fold more abundant in bloodstream trypomastigotes than in procyclic forms, as expected from measurements of enzyme activity. This regulation is at the level of transcription. Genomic mapping and cloning results indicate that trypanosomes have four copies of the aldolase gene arranged as two copies of a tandem repeat. The deduced protein sequence has a short N-terminal extension (relative to other known aldolases) which could be involved in the glycosomal localization of the enzyme.

Transcriptional and sequence data will also be presented for an RNA which is abundant in procyclic but not bloodstream trypanosomes.

C35 CHROMOSOME SIZE POLYMORPHISMS IN **PLASMODIUM FALCIPARUM** CAN INVOLVE DELETIONS AND OCCUR FREQUENTLY IN NATURAL PARASITE POPULATIONS.

Lynn M. Corcoran, Karen P. Forsyth A. Edward Bianco, Graham V. Brown and David J. Kemp

We have used the recently developed procedure of pulsed-field gradient electrophoresis to study genomic organization in **P.falciparum**. This parasite's genome comprises a small number (>7) of chromosomes, ranging in size from ~800 to several thousand kb. A comparison of molecular karyotypes of different **P.falciparum** isolates that have been maintained **in vitro** revealed that chromosome number was constant, whereas the sizes of analogous chromosomes varied markedly, sometimes by more than 30% between isolates. We show here that the molecular karyotype of a cloned parasite line is constant through the asexual blood stages. Thus the morphological changes that occur during this part of the life cycle are not accompanied by karyotypic changes. To examine chromosome size polymorphisms between two independent parasite populations, we used whole **P.falciparum** chromosomes as hybridization probes. The results indicate that these polymorphisms are not the consequence of large interchromosomal exchanges, but imply that deletions/duplications represent one mode of generating chromosome length polymorphisms. Although such deletions may primarily involve losses of repetitive DNA, in some cases structural genes for **P.falciparum** antigens can also be lost. Furthermore, these dramatic chromosome size polymorphisms occur not only **in vitro** in cultured lines of **P.falciparum**, but they occur with remarkable frequency in natural malarial infections.

C36 DEVELOPMENTALLY REGULATED SCHISTOSOME GENES AND ANTIGENS,

John S. Cordingley,* Kevin Johnson, and David W. Taylor, Department of Pathology, University of Cambridge, Cambridge, ENGLAND CB2 1QP. *Current address: Department of Biochemistry University of Wyoming, Laramie, Wyoming 82071

We have isolated and sequenced cDNA clones of several developmentally regulated genes from Schistosoma mansoni. One antigen is the major protein in eggs and miracidia and its mRNA forms approximately 2% of the total in these stages. The complete sequence shows homologies with developmentally regulated genes from Drosophila and enables several possible functions for the protein to be suggested.

Another gene encodes a major protein of the vitellaria and is expressed only in mature female worms. It has very strong homologies to silk moth chorion genes ($p<10^{-9}$) and is probably an eggshell component. Only one copy is present in the genome.

C37 CORRELATION OF TRICHINELLA DNA STRUCTURE WITH PIG INFECTIVITY

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Isolates of Trichinella from sylvatic hosts differ in their potential to reproduce in pigs. The structure of the genomic DNA from more than 10 isolates, including T. spiralis, T. nativa, T. pseudospiralis, and previously uncharacterized Trichinella isolates, has been examined and correlated with the potential of the isolate to infect pigs. Restriction endonuclease Hind III fragments of genomic DNA separated by agarose gel electrophoresis and stained with ethidium bromide exhibit banding patterns of repetitive DNA fragments which are characteristic of the isolate. Four basic patterns have been identified to date. Three of these are correlated with the three major types of Trichinella mentioned above. The fourth is represented by two isolates from North American carnivores. The DNA of sylvatic isolates which readily infect pigs produces in this analysis the same banding pattern as DNA from the T. spiralis isolates from domestic pigs. Sylvatic isolates with any one of the other three patterns have <1% of the reproductive capacity in pigs of the T. spiralis isolates. Two clones of repetitive DNA from T. spiralis (Beltsville Pig Strain), BP1 and BP2, were selected from a library of Sau3A genomic DNA fragments in pUC8. When used as probes these clones hybridized only to the DNA of isolates which readily infect pigs. Therefore, clones BP1 and BP2 are potentially useful for predicting whether new isolates will be infectious for pigs.

C38 CHARACTERIZATION OF AMPLIFIED DNA FROM TUNICAMYCIN RESISTANT LEISHMANIA MEXICANA.

S. Detke, J. A. Kink and K.-P. Chang, Department of Microbiology and Immunology, University of Health Sciences, The Chicago Medical School, North Chicago, IL 60064

Acclimatization of Leishmania mexicana amazonensis (LV78) to increasing drug concentrations made possible the production of promastigote clones resistant to 80 ug/ml tunicamycin, an inhibitor of N-acetylglucosaminyltransferase. This enzyme increases in activity in the drug-resistant cells as compared to the wild-type, but remains tunicamycin-sensitive. Restriction endonuclease digestion of the DNA of the mutant cells gave discrete fragments which were not visible in the wild-type cells. We used twelve different restriction nucleases and in each case found two or more bands of DNA which were inapparent in the wild-type cells. The total length of the amplified DNA is about 66 kb in size and appears to be repeated 30 to 50 times in the drug-resist cells. We have cloned two-thirds of this amplified DNA using BamH I restriction fragments and pBR-322 in three plasmids, containing 12, 14.5 and 15 kb of the amplified DNA, respectively. Dot blot analysis using cloned DNAs as probes agrees with the estimate of a DNA amplification of 30 to 50 fold in the mutants. Although restriction mapping of these inserts with Ava I suggest some homology between the 14.5 and 15 fragments, no cross hybridization was observed between these fragments and between any of these and a 22 kb fragment, which represents the remaining one-third of the amplified region and is currently being cloned. (Supported by NIH-NIAID grant #AI20486 to KPC).

C39 ISOLATION OF ENTAMOEBA SURFACE ANTIGEN GENES, Ursula Edman, Isaura Meza*, Marcos Rojkind*, Alfredo Lopez* and Nina Agabian, Naval Biosciences Laboratory, University of California, Berkeley, 94720. *Centro de Investigacion y de Estudios Avanzados del IPN, Mexico D.F. In order to contribute to the understanding of the mechanism of differential virulence in Entamoeba histolytica, we have isolated several surface antigen genes as potential genetic markers of virulence. cDNA expression libraries were constructed from strains of intermediate and high virulence, HM1:IMSS and HM38:IMSS respectively, and were screened with mouse and rabbit antisera prepared against membrane fraction isolated from HM1:IMSS. These antisera recognize seven polypeptides ranging in molecular weight from 20 to 200kD and include a carbohydrate-binding polypeptide as well as an erythrocyte lysis activity. The isolated clones are being characterized by Northern analysis, sequence determination, preparation of monospecific antisera and immunofluorescence.

C40 THE BENT HELIX IN CRITHIDIA KINETOPLAST MINICIRCLES, P. Englund*, P. Kitchin*, C. Rauch*, K. Ryan*, D. Kang†, R. Wells†, M. Bleyman# and J. Griffith#, *Johns Hopkins Medical School, Baltimore, MD 21205, †University of Alabama at Birmingham, Birmingham, AL 35294, and #University of North Carolina, Chapel Hill, NC 27514

Kinetoplast minicircles from most trypanosomatid species have a region of bent helix. Restriction fragments containing the bend have anomalously low electrophoretic mobility in polyacrylamide gels and low rotational relaxation times. A survey of several trypanosomatids revealed that the most extremely bent helix, as judged by gel electrophoresis, is from Crithidia fasciculata. Analysis of a cloned Crithidia minicircle revealed that the bend is localized in a region of about 200 bp; we subcloned this region as a 213 bp insert in plasmid pPK201/CAT. This 213 bp fragment has unusual properties: (1) It migrates much slower on polyacrylamide gels than expected for its size. (2) It has unusual circular dichroism spectra. In aqueous buffer the spectrum resembles that of B DNA, but with increasing alcohol (between 44 and 51%) it changes dramatically indicating a structural transition. The spectrum in high alcohol resembles that of neither A, B, C, nor Z DNA. (3) As viewed by electron microscopy, the fragment is highly curved. The 213 bp fragment contains 18 runs of 4 to 6 A's, and 16 of the runs are in the same strand. Many A-runs are spaced with a periodicity of one per helical repeat. As suggested for another kinetoplast fragment (Wu and Crothers, Nature 308, 509, 1984), the bending is probably due to the cumulative effect of small bends periodically positioned along the helix. These small bends could occur at junctions between different helical structures in the A-runs and in the intervening sequences.

C41 TRYPANOSOMA BRUCEI MITOCHONDRIAL GENES HAVE MULTIPLE TRANSCRIPTS, SOME OF WHICH ARE DIFFERENTIALLY EXPRESSED DURING THE LIFE CYCLE, Jean E. Feagin, Douglas P. Jasmer, and Kenneth Stuart, Issaquah Health Research Institute, Issaquah, WA 98027

Bloodstream forms (BF) of T. brucei lack cytochromes and a Krebs cycle; upon differentiation to procyclic forms (PF) a fully functional respiratory system is elaborated. We have compared mitochondrial (maxicircle) DNA transcripts in these stages to examine the basis for this differential expression of the respiratory system during the life cycle. All maxicircle genes are transcribed in both life cycle stages and most have multiple transcripts. Each putative protein coding gene, with one exception, has two major transcripts differing in size by 150-200 nt. The size difference between transcripts from a single gene is probably not due to polyadenylation since all these transcripts are present in poly(A)$^+$ RNA. The location and nature of the extra nucleotides are under investigation. Some transcripts are similar in abundance between the two life cycle stages but others differ. The larger of the two transcripts for cytochrome c oxidase I and II and apocytochrome b are more abundant in PF than BF while the single NADH dehydrogenase subunit 5 transcript is more abundant in BF. The variability of transcript abundances during the life cycle, including independent variation for transcripts from the same gene, implies at least some individual modulation. Differential expression of maxicircle transcripts is probably mediated posttranscriptionally.

C42 CHARACTERIZATION of the MINI-EXON PRECURSOR RNA of TRYPANOSOMA BRUCEI, Marion S. Freistadt and George A. M. Cross; Laboratory of Molecular Parasitology; and Andrea D. Branch and Hugh D. Robertson; Laboratory of Genetics; The Rockefeller University, 1230 York Ave., New York, New York 10021

Many parasitic kinetoplastids appear to form their mRNA by a novel mechanism involving discontinuous transcription. A 35-nucleotide RNA segment (the "mini-exon") is found at the 5' terminus of many, if not all, messenger RNAs. However, DNA encoding the mini-exon sequence is chromosomally unlinked to any known structural gene. Indirect analyses have predicted the existence of a 140-nucleotide RNA ("medRNA" or SL RNA") containing the same 35 nucleotides at its 5' end. This RNA is likely to be the source of the mini-exon in mRNA.

[^{32}P] *in vivo* labelling of T. brucei RNA, hybrid-selection of labelled mini-exon sequences and size fractionation from polyacrylamide gels have been used to isolate the mini-exon precursor as well as other higher and lower molecular weight RNAs containing regions complementary to mini-exon genomic DNA. RNAse fingerprinting has been used to confirm the identity of the mini-exon precursor RNA. A nuclease resistant product with mobility characteristic of a cap structure has been detected. Properties, such as the cap structure, of these RNAs will be described.

C43 IDENTIFICATION OF A TELOMERIC DNA SEQUENCE IN Plasmodium berghei, Clara Frontali, Marta Ponzi, Elisabetta Dore, Tomasino Pace, Istituto Superiore di Sanità, Rome, Italy.

The present study was undertaken in order to test whether the plasmodial genome is organized in linear molecules, whose termini, one expects, would exhibit the peculiar features described for other lower eukaryotes (tandem reiteration of a short basic repeat initiating with a cluster of cytosine residues; presence of single strand discontinuities; terminal hairpin). As a consequence telomeric regions would be excluded from any genomic library not especially designed to include them.
The strategy we adopted for the construction and screening of a suitable library from P. berghei DNA allowed the selection of a recombinant clone which recognizes in total genomic digests bands which are sensitive to digestion with Bal31 exonuclease. The distal portion of the cloned insert consists of the reiteration of the heptanucleotide CCCTGAA where the G is irregularly substituted by an A. At least 70 repeats are present.
The composition of the basic repeat involving all the four nucleotides justifies the results of the selective radiolabelling at endogenous single strand gaps.
The telomeric insert recognizes Bal31 sensitive bands also in other Plasmodium species. It hybridizes to all the chromosome-sized bands revealed by pulsed field gradient electrophoresis.

C44 MOLECULAR CLONING AND CHARACTERIZATION OF DEVELOPMENTALLY REGULATED GENES IN Trypanosoma cruzi. Constantine G. Haidaris, Guenter Harth and Magdalene So. Research Institute of Scripps Clinic, La Jolla, CA 92037.

Trypanosoma cruzi, the causative agent of Chagas' disease, undergoes several morphological changes during its life cycle. Conversion from non-infectious epimastigote (EPI) to infectious trypomastigote has been linked to structural, biochemical and antigenic changes in this organism. These changes are likely to be based on differential gene expression in the two forms. We sought to isolate genes preferentially expressed in the trypomastigote in the hope of identifying determinants involved in the infection of host cells by trypomastigotes. We used the technique of subtraction hybridization cloning to acheive this goal. Poly A+ mRNA was purified from EPI and cultured metacyclic trypomastigotes (MT) and single stranded cDNA was prepared from the MT poly A+ mRNA. The MT cDNA was hybridized with an excess of EPI mRNA. The common sequences between EPI and MT form heteroduplexes and the MT unique cDNA species remain unhybridized. The hybridization mix was passed through hydroxyapatite which binds the heteroduplexes and allows the single stranded species to flow through, thereby providing a population of molecules enriched MT unique species. Double stranded cDNA was prepared from this pool and cloned into pUC19. Upon transformation into E. coli, 30,000 clones were obtained and 5000 were screened on replicate filters using radiolabelled mRNA from EPI and MT as a probe. After 4 rounds of screening, approximately 40 clones were identified whose mRNA was preferentially expressed in MT by at least 10 fold based on signal intensity. This was confirmed by Northern blotting using purified plasmids from several clones. We are presently working on expression of these isolates.

C45 IN VITRO PACKAGING OF KINETOPLAST DNA NETWORKS, S.L. Hajduk and L.S. Silver, University of Alabama at Birmingham.

The mitochondrial DNA of trypanosomes is organized as a complex network of catenated circular DNA molecules. The kDNA network of Trypanosoma equiperdum consists of 5000 small (1kb) circular molecules mincircles, and about 50 copies of a larger circular DNA (14kb) called the maxicircle. All minicircles contain a region of DNA in a "bent" helical conformation. The function of the minicircles and the bent region of DNA is unknown. Isolated kDNA networks are large open structures while the kDNA in situ appears to be a highly compacted and regularly arranged structure. Low concentrations of polyamines (4-5mM) can condense DNA in the absence of proteins. We have used spermidine to compact kDNA networks T. equiperdum in order to determine whether compacted kDNA is folded into a regular structure. Two assays have been developed to examine the organization of these in vitro compacted networks. 1) When kDNA networks are compacted with spermidine and then digested with the restriction endonucleases HinfI and CfoI, which have sites about 500 bp apart on the 1kb minicircle, only the CfoI site is susceptible to cleavage. The HinfI site is completely protected. Uncompacted, nicked and linear kDNA and compacted pBR322 DNA are all sensitive to cleavage by HinfI. 2) Treatment of compacted kDNA networks with DNAseI preferentially nicks the minicircles near the CfoI site. These results indicate that compactation of the T. equiperdum kDNA network with spermidine results in a regular alignment of the minicircles and we suggest that this regular packaging is directed by the bent region of the minicircles.
Supported by NIH AI 21401.

C46 CHARACTERIZATION OF THE ACTION OF MUNG BEAN NUCLEASE ON AFRICAN TRYPANOSOME GENOMIC DNA. Kim Henkle-Brown, Steven T. Brentano and John E. Donelson, University of Iowa, Iowa City, IA, 52242

Trypanosome telomere-linked variable surface glycoprotein (VSG) genes have been difficult to clone because their flanking regions frequently lack restriction sites. Therefore, we have constructed a recombinant DNA library of fragments generated by mung bean (MB) nuclease - an enzyme which cleaves before and after genes in Plasmodium DNA (McCutchan et.al., Science 225;625 (1984)). Southern hybridizations with several gene probes showed that under the appropriate conditions MB nuclease produces discrete trypanosome DNA fragments that are as clearly resolved on an agarose gel as restriction fragments. The majority of VSG genes are on fragments of about 1.7 kb. A library of MB nuclease generated fragments was constructed and screened with two specific VSG genes to examine the site of MB nuclease action. Eight clones were sequenced and the MB nuclease cleavage sites were mapped. In general, MB nuclease cleaved 300-800 bp in front of the VSG start codon and within 50 bp on either side of the termination codon. Both telomere-linked VSG genes and interior basic copy VSG genes were contained in the library. Therefore, the library potentially contains all of the telomere-linked VSG genes in the genome.

C47 EXPRESSION OF MITOCHONDRIAL AND NUCLEAR GENES DURING DIFFERENTIATION OF AFRICAN TRYPANOSOMES, George C. Hill, Gina Benavides, Soma Chaudhuri, and Desiree Sylvester Meharry Medical College, Division of Biomedical Sciences, Nashville, TN 37208

African trypanosomes undergo differentiation from bloodstream to procyclic trypomastigotes as they pass from the vertebrate to the insect vector. We have been investigating the molecular and biochemical changes that occur during development of the mitochondrial electron transport system. We have cloned and sequenced from Trypanosoma brucei the apocytochrome b gene which is encoded by maxicircle DNA and are determining the levels of transcription of this gene in both bloodstream and procyclic trypomastigotes. Similar studies are being undertaken with cloned COII and apocytochrome c genes, encoded by maxicircle DNA, and nuclear DNA respectively. We are interested in the kinetics and factors affecting appearance of these transcripts during the initial stages of differentiation. Our initial results suggest that as early as 36 hours after differentiation is initiated, an increase in transcripts for apocytochrome b and COII occurs. To further these studies, two synthethic peptides covering hydrophilic regions of the protein (amino acid residues 239-250 and 251-272) have been synthesized from the amino acid sequence inferred from the nucleotide sequence of apocytochrome b. The purification of T. brucei cytochrome b from a cytochrome bc_1 complex, is also being undertaken, taking advantage of the unique properties of cytochrome b in nonionic detergents. In addition, studies on the transcription and translation of apocytochrome c are also underway.

C48 ISOLATION, CLONING, AND EXPRESSION OF A PROTEASE FROM ANCYLOSTOMA HOOKWORMS, Peter Hotez, George Newport, N.L. Trang, Nina Agabian, and Anthony Cerami, Rockefeller University, New York, NY 10021, and University of California, Berkeley CA 94625

The adult hookworm Ancylostoma caninum was shown to release a 37 kDa protease which was essential for its adaption to parasitism. The enzyme was purified from parasite extracts by ion-exchange, gel-filtration, and hydrophobic-interaction chromatographies and shown to have an N-terminal sequence of R-H-H-Q-P-K-A-L-L-G-A-H-G-G-I. The protease functions as an elastase similar to those found in hemorrhagic snake venoms. Antiserum was prepared against the purified enzyme and used to screen a cDNA library from hookworm RNA prepared in the expression vector λgt11. A positive clone was identified and used to produce lysogens which upon induction with IPTG expressed a 126 kDa β-galactosidase fusion polypeptide. The hybrid protein was purified by sucrose cushion centrifugation and preparative SDS-PAGE and used in an enzyme immunoassay to demonstrate anti-protease antibodies from hookworm infected dogs. Serum from an infected dog showing monospecificity to the 37 kDa protein on western blots cross-reacted to the hybrid protein on enzyme immunoassay, confirming the identity of the clone. Endonuclease digestion of the recombinant phage with EcoRI revealed an 800 bp insert which was mapped, subcloned into M13 and dideoxy sequenced; an open reading frame was obtained. The translated sequence exhibited a large internal stretch of hydrophobic residues and positively charged hydrophilic residues at opposite ends of the polypeptide; a finding consistent with the behavior of the native protein on chromatography.

C49 CHARACTERIZATION OF A HISTIDINE-RICH PROTEIN RELATED GENE FROM PLASMODIUM FALCIPARUM, David O. Irving, Mary Morry and George A.M. Cross, The Rockefeller University, New York, NY 10021

A Plasmodium lophurae histidine-rich protein (HRP) cDNA probe was used to screen a partial Sau 3A genomic library derived from P. falciparum DNA. A single clone of 2.5 kb (Pf1) was isolated which hybridized strongly to a species > 6.0 kb on Southern blots of mung bean nuclease digested P. falciparum DNA and to a band of similar size on Northern blots of P. falciparum RNA. However, there was only weak cross-hybridization of Pf1 to P. lophurae HRP mRNA. This implied that, unlike the P. lophurae HRP gene, the P. falciparum gene from which Pf1 was derived does not encode a protein consisting of tandemly repetitive histidine residues. Nucleotide sequence analysis of the cloned DNA supported this. Experiments were designed, therefore, to try to determine the function of this gene in P. falciparum.

Southern blot analysis of restriction digests of DNA from four different geographic isolates of P. falciparum using Pf1 as a probe, indicated that the gene is conserved. Northern blots of RNA isolated from P. falciparum at various stages of the intraerythrocytic cycle indicated that there is some developmental regulation of transcription of this gene as the mRNA appeared to be present predominantly late in the cycle. Thus, the protein encoded by this gene may play some role in invasion. To investigate this further, rabbit antisera are presently being raised against fusion proteins constructed by ligating various fragments of Pf1 to the 5' region of the E. coli trp E gene. These will be used for immunofluorescence assays and Western blot analyses.

C50 MAXICIRCLE TRANSCRIPTS DIFFER AMONG ISOLATES OF TRYPANOSOMA BRUCEI, Douglas P. Jasmer, Jean E. Feagin, and Kenneth Stuart. Issaquah Health Research Institute, Issaquah, WA 98027.

The maxicircle of T. brucei contains gene sequences for rRNAs, cytochromes, components of the NADH dehydrogenase complex and probable protein coding genes whose function remains to be elucidated. These genes are interspersed by sequences of unknown function many of which are G+C rich and exhibit strong G versus C strand bias. These sequences encode G rich transcripts. We have compared RNA from 8 isolates of T. brucei subpecies. Although transcripts from rRNAs and putative protein coding genes do not differ among isolates, four of the G+C rich sequences encode multiple transcripts that vary in size among these isolates. These transcript differences are isolate dependent, and thus are not correlated with subspecies designations. These transcript differences may reflect transcript processing events.

C51 PURIFICATION AND CLONING OF TOXOPLASMA GONDII TACHYZOITE DNA, Alan M. Johnson, J. P. Dubey, and John B. Dame, USDA/ARS, Animal Parasitology Institute, Beltsville, Maryland, 20705

Although it is possible to obtain small amounts of pure T. gondii DNA from the oocyst stage found in cat faeces, most research on T. gondii is performed using the proliferative stage, the tachyzoite. Large numbers of tachyzoites of the RH strain of T. gondii may be easily grown in the peritoneal cavity of mice, but this is a problem for nucleic acid analysis of the parasite. Tachyzoites are rarely obtained greater than 99% pure, and the large difference in genome size results in a DNA preparation which is greater than 50% murine DNA. To overcome this problem tachyzoite DNA (RH strain) was separated from murine DNA by isopycnic density gradient ultracentrifugation in CsCl containing the fluorescent dye Hoechst 33258. This dye binds to A+T rich regions of DNA, thereby allowing the separation of parasite and murine DNA based on the differing A+T content. Quantitative hybridization studies with mouse spleen DNA and with T. gondii DNA extracted from oocysts of the ME49 strain confirmed that tachyzoite DNA could be obtained at least 90% pure with a recovery of at least 90%. A mung bean genomic library of purified tachyzoite DNA was prepared in λgt11 and to date the recombinants have been screened with RNA from the closely related protozoan Eimeria acervulina, and the T. gondii rRNA genes have been isolated.

C52 Sequence analysis of the WRATAT 1.1 VSG, progenitor to a series of metacyclic VSGs in Trypanosoma brucei rhodesiense. Gregory Kelly, Klaus M. Esser*, and John E. Donelson, Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242, and Walter Reed Army Research Institute*, Washington, D.C. 20012.

We have previously described the cloning and analysis of two metacyclic VSG genes [Lenardo, M.J., et.al. PNAS 81 6642-6646 (1984)]. During the metacyclic developmental stage, trypanosomes express a restricted subset of variant antigen types, in contrast to the plethora of surface antigen types available to the organism while in the mammalian bloodstream. We have cloned and sequenced a cDNA encoding the WRATAT 1.1 VSG. WRATAT 1.1 was the antigen being expressed by the trypanosomes which generated the metacyclic repertoire of VSGs under investigation in our laboratories. One of us (K.M.E.) has identified a re-expressor of the WRATAT 1.1 surface antigen (WRATAT 1.19). This re-expressor is a blood stream VSG which appears early in the course of infection with WRATAT 1.1 derived metacylic organisms. We have used our cDNA clone for the WRATAT 1.1 VSG to examine the structure, location, and expression of the gene for this antigen type in both WRATAT 1.1 and 1.19 trypanosomes. These results will be discussed in the context of our continuing effort to understand the restricted expression of metacyclic VSG genes.

C53 THE TRANSCRIPTION UNIT OF AN IN SITU ACTIVATED VARIANT SURFACE GLYCOPROTEIN(VSG) GENE IN T.b.brucei IS AT LEAST 45 kb AND YIELDS MULTIPLE STABLE mRNAs. Jan M.Kooter, Ron Wagter, Nel van Harten-Loosbroek, Arend Jan Winter and Piet Borst, The Netherlands Cancer Institute, Plesmanlaan 121, 1066CX Amsterdam

Antigenic variation in trypanosomes is achieved via consecutive activation of VSG genes.Chromosome internal genes are activated by way of a duplicative transposition to a telomeric expression site.Transcription starts far upstream of the duplicated segment and these genes are therefore activated by promoter addition(1). VSG genes already located at chromosome ends can be activated in situ, without detectable rearrangements close to the gene. Analysis of nascent RNA reveals that the in situ activated VSG gene 221 and at least 45kb of upstream sequences are transcribed at about constant rate in isolated nuclei . Transcription is α-amanitin resistant, a characteristic of VSG gene transcription (2),and absent in culture form trypanosomes. These results suggest that VSG pre-mRNA synthesis initiates at least 45 kb upstream of the gene(3). Besides VSG mRNA, the transcription unit yields several minor mRNA-like RNAs with open reading frame. Most of the DNA segments of this unit hybridize with multiple bands in Southern blots even at high stringency. We conclude that there are multiple,highly conserved telomeric expression sites for VSG genes in trypanosomes. (1) De Lange,T. et al.(1985) EMBO J.,in press, (2) Kooter,J.M. and Borst,P. (1984) Nucl.Acids Res. 12,9457-9472, (3) Bernards, et al.(1985) Mol.Cell.Biol. 5, 545-553.

C54 STRUCTURE AND EXPRESSION OF TUBULIN GENES IN LEISHMANIA ENRIETTII, Scott M. Landfear and Dyann F. Wirth, Harvard School of Public Health, Boston, MA 02115

Recently, we have cloned the α- and β-tubulin genes from Leishmania enriettii. These are especially interesting genes, because they encode the two most abundant proteins of the organism, and because their expression is regulated during the life cycle of the parasite. As these are the first nuclear genes from Leishmania whose structure has been studied in detail, we have identified potential promoter sequences, and we have studied the synthesis and processing of the tubulin mRNAs. The sequence of the α- and β-tubulin genes upstream from the region coding for mature mRNA has been determined, and homologies have been identified which may be important in promotion or coordinate regulation of transcription. Sequencing of the 5' ends of the tubulin mRNAs indicates that they contain a 35 nucleotide spliced leader sequence not encoded within the tandemly repeated tubulin genes. This spliced leader is probably added onto the mRNA either by a novel transcriptional priming event, or by a novel trans-splicing reaction.

C55 IDENTIFICATON OF A TRYPANOSOMA B. RHODESIENSE RNA TRANSCRIPT PRESENT ONLY IN HUMAN SERUM RESISTANT VARIANTS. G. Matthyssens, C. De Greef, N. Van Meirvenne, and R. Hamers, Free University Brussels, Belgium.

Normal human serum is cytotoxic for T.b. brucei but not for the human infective forms. This host range specificity therefore differentiates T.b. brucei from both T.b. rhodesiense and T.b. gambiense, the causative agents of human sleeping sickness. The biochemical nature of this lysis remains to be elucidated. If one hypothesizes that resistance to lysis is mediated by membrane changes either at the structural or enzymatic level, we should be able to detect a specific transcript(s) in a serum resistant trypanosome variant which is absent in the serum sensitive forms. Highly labeled cDNA was made from poly A^+ RNA derived from resistant T.b. rhodesiense and hybridized to poly A^+ RNA from the sensitive variant. Non-hybridized cDNA was used as probe on Northern blots and was found to hybridize with a 1.45 kb transcript present in RNA from serum resistant variants only.

C56 DEVELOPMENTAL REGULATION OF MITOCHONDRIAL RIBOSOMAL RNA TRANSCRIPTION IN TRYPANOSOMES, E.F. Michelotti and S.L. Hajduk, University of Alabama at Birmingham.

The developmental stages of T. brucei found in the insect vector have a fully functional mitochondrion. The bloodstream trypanosomes completely supress mitochondrial biogenesis and survive entirely by glycolysis. We have analyzed transcription of the 9S and 12S mitochondrial ribosomal RNA genes by northern blot and quantitative S_1 analysis. Results suggest that rRNA transcription is reduced 30-fold in the early bloodstream trypanosomes compared with the later bloodstream stages. Differentiation of the late bloodstream trypanosomes to the insect developmental stages results in less than a two-fold increase in rRNA transcription. We have also found that mitochondrial rRNA transcription remains suppressed in trypanosomes passaged at 2-3 day intervals in mice for 8 days. Trypanosomes maintained in a single animal for 8 days had 6-fold higher levels of rRNA. It appears that the trypanosome mitochondrial rRNA transcription is responding either to a host factor which represses mitochondrial rRNA transcription and is depleted during the trypanosome infection, or to a positively acting parasite or host factor which accumulates during the bloodstream infection. The increase in rRNA transcription is reversible. Mitochondrial rRNA transcription was measured in early infections initiated with late bloodstreat trypanosomes which show high levels of mitochondrial rRNA transcription. The level of transcription is reduced 6-10-fold in these infections. Supported by NIH AI 21401.

C57 CLONING AND CHARACTERIZATION OF A GENE INVOLVED IN DISCONTINUOUS TRANSCRIPTION, Samuel I. Miller, Scott Landfear and Dyann F. Wirth, Harvard School of Public Health, Boston, MA 02115.

Studies on L. enrietti tubulin genes indicated a 35 base pair addition to the 5' end of messanger RNAs. The gene that codes for this 35 base pair addition has been cloned and sequenced. It exists in the genome as a tandem repeat of 438 bases of approximately 150 copies. The sequence of this gene has homology with the spliced leader sequence of other kinetoplastida only over the 35 base pair addition and the regions just 5' and 3' to this added sequence. Northern analysis demonstrates that this gene codes for a transcript of approximately 80 bases and that this gene hybridizes to many leishmania RNAs. Primer extension and S_1 analysis demonstrate that this gene's 80 bp transcript contains the 35 bp addition on the 5' end. Primer extension sequencing of the alpha and beta tubulin RNAs demonstrate this 35 bp addition on the 5' end of messenger RNA.

C58 THE MONOGENETIC TRYPANOSOME, CRITHIDIA FASCICULATA, CONTAINS A TRANSCRIPTIONALLY ACTIVE, MULTICOPY MINI-EXON SEQUENCE, Michael L. Muhich, Dallas Hughes, Aqda M. Simpson and Larry Simpson, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

A repeated sequence from the C. fasciculata nuclear genome has been isolated which is homologous to the Trypanosoma brucei mini-exon gene. Sequence analysis of the 420 bp monomeric unit confirms the presence of a 35 nucleotide sequence within the repeat that is 86% (30 out of 35 nucleotides) homologous with the T. brucei mini-exon sequence. The repeat is present at approximately 250 copies per cell and is organized into one, or a few, large head to tail tandem clusters predominantly on a single chromosome. Transcription of the mini-exon repeat gives rise to a major 82 nucleotide, and a minor 85 nucleotide poly(A)- RNA, the first 35 nucleotides of which comprise the mini-exon sequence. The 3'-termini of the steady state transcripts map to a posistion on the DNA sense-strand directly preceeding or within a stretch of 8 thymidine residues.

C59 ANALYSIS OF TUBULIN GENES IN *TOXOPLASMA GONDII*, Susi D. Nagel and John C. Boothroyd, Stanford University, Stanford, CA 94305.

The α and β tubulin genes have been isolated from a λgt11 genomic library of *Toxplasma gondii*. These genes are being used to study the molecular biology of toxoplasma and to determine whether discontinuous transcription occurs outside members of the Kinetoplastida family. Southern analysis shows that toxoplasma has a single copy of both the α and β tubulin genes, in contrast to most other eukaryotes where tubulins exist as multigene families. The sizes of both mRNAs as determined from Northerns are about 2.3 Kb each. Partial sequencing has revealed the presence of at least three introns within the coding region of the β gene and at least one intron in the α coding region. The interruption of a coding sequence by introns has not been previously described in genes of parasitic protozoa. The β gene has a 464 base pair intron after the first 32 amino acids and introns of 141 and 99 base pairs near the termination codon. Its deduced amino acid sequence is 91% homologous to mammalian β tubulins, and 87.5% homologous to trypanosome β tubulin. Experiments in progress include mapping of the 5' end of these genes by S1 and primer extension, as well as further sequencing.

C60 A REPLICATION ORIGIN IN TRYPANOSOMA EQUIPERDUM KINETOPLAST MINICIRCLES, James Ntambi, Kathleen Ryan and Paul Englund, Johns Hopkins Medical School, Baltimore, MD 21205

In Trypanosoma equiperdum, some newly replicated kinetoplast DNA minicircles contain a small gap, of roughly 10 nucleotides, in the newly synthesized strand. This gap overlaps the GGGGTTGGTGTAA sequence found in all minicircles, from several species, which have been sequenced (J. Biol. Chem. 260, 5574, (1985)). We have found that ribonucleotides are associated with this gap. The evidence for ribonucleotides includes: (1) alkali lability of minicircles in which the gap had been repaired by T4 DNA polymerase and T4 DNA ligase; (2) susceptibility of alkali-treated minicircles to phosphorylation with polynucleotide kinase and γ[^{32}P]ATP; (3) detection of [^{32}P]ribonucleotides on DNA which had been dephosphorylated with phosphatase, rephosphorylated with polynucleotide kinase and γ[^{32}P]ATP, and then hydrolyzed with NaOH. Using sequencing methodology, we mapped the ribonucleotides to the 5' end of the newly synthesized strand. There are one or two ribonucleotides present, and the RNA:DNA junction is at one of two neighboring positions in the DNA sequence. It is likely that these ribonucleotides are remnants of a replication primer. It is possible that most of the primer had already been excised, to create the gap, and that only a remnant remained. Subsequent processing would release these remaining ribonucleotides. The presence of ribonucleotides at the 5' end of the newly synthesized strand provides strong evidence that the GGGGTTGGTGTAA sequence is conserved in minicircles of all species because it is a replication origin.

C61 CLONING OF AN ONCHOCERCA SPECIFIC REPEATED DNA SEQUENCE, Francine B. Perler, Marta Meda, Mike Casasanta, New England Biolabs Inc., Beverly, Ma. 01915, and Marc Karam, OCP, Ouagadougou, Burkina Faso.

The filarial nematode, Onchocerca volvulus, infects over 40 million people and is one of the major causes of world blindness. WHO, through OCP, has been trying to control Onchocerciasis in West Africa by eliminating the black fly vector. Epidemiological work is hampered by the absence of a fast and easy method for detecting and identifying O.volvulus in human, animal, and insect populations.

We have cloned repeat genes from O.volvulus, which may lead to a rapid biochemical assay for Onchocerca. pOV26 can detect one L3 infective larvae in dot blot experiments. It is specific for Onchocerca species, and doesn't cross react with human or other filarial DNAs.

pOV8 contains an O.volvulus repeat sequence which cross reacts with all filarial worms tested(7), but does not cross react with host DNA (human or cow). Although this clone can not be used to detect O.volvulus specifically, it may be useful as a first line probe for filarial infection in general.

We have also used our DNA probes to examine the question of whether virulence differences observed in forest vs. savannah regions of West Africa are due to strain differences or other factors. Preliminary results indicate no restriction enyzme polymorphism between DNA isolated from forest worms or savannah worms, when Southern blots were probed with 2 different cloned O.volvulus repeat sequences, and O.volvulus rRNA genes.

In summary, we have cloned 2 DNA repeats which we are examing as potential DNA species probes.

C62 CHARACTERIZATION OF A HISTIDINE RICH PROTEIN GENE FROM A KNOBBY STRAIN OF PLASMODIUM FALCIPARUM, Laura G. Pologe and Jeffrey V. Ravetch, Sloan-Kettering Institute, New York, N.Y. 10021

cDNA clones from a P. falciparum FcR-3 library have been isolated. These clones hybridize to an mRNA species which is transcribed in knobby strains but not in knobbless variants of those strains. Sequence analysis reveals that this gene encodes multiple tandemly repeated stretches of histidine residues. The structure of this gene in its local context as well as in a chromosomal context in both knobby and knobbless strains is currently under investigation using Southern blot analysis and pulsed-field electrophoresis. These studies are aimed at describing the molecular basis for the switch from the K+ to the K- phenotype for this gene.

C63 ISOLATION OF A FEMALE-SPECIFIC cDNA CLONE FROM SCHISTOSOMA MANSONI, D. Rekosh, L. Bobek, H. van Keulen and P.T. LoVerde, State University of New York at Buffalo, Buffalo, N.Y. 14214

We have isolated a female-specific cDNA clone by the technique of subtractive hybridization. mRNA was extracted from sexually-mature female worms and ^{32}P-labelled single-stranded cDNA was synthesized. After hydrolysis of the RNA, the remaining cDNA was hydridized to a ten molar excess of mRNA extracted from male worms. cDNA sequences remaining single stranded were shown to be female-specific using RNA dot blots. This cDNA probe was used to screen an adult worm cDNA library. A series of plasmids from positive colonies were isolated. One of these female-specific clones was chosen for further study. mRNA which hybridizes to this clone is found only in mature female worms. Virtually no sequences complementary to it can be found in adult male worms, eggs or immature females from unisexual infections. During a normal bisexual infection of hamsters, complementary mRNA sequences first appear at 30 days post infection, a time which coincides with the pairing of the worms. We are currently determining the sequence of this clone and carrying out experiments to test the hypothesis that the expression of this gene in female worms is regulated by the presence of males.

C64 kDNA SEQUENCE EVOLUTION AND THE CHARACTERIZATION OF LEISHMANIA POPULATIONS Rogers, W.O., Lopes, U.G. and Wirth, D.W. Department of Tropical Public Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA, 02115

Kinetoplast DNA, the mitochondrial DNA of kinetoplastid protozoa, has been used for the identification of Leishmania populations and for the diagnosis of Leishmaniasis. Shotgun cloning of kDNA fragments was used to isolate sequences of varying taxonomic specificities. Fragments specific for all visceral Leishmania species, for both African and American strains of visceral Leishmania, and for a single isolate have been cloned. Similar experiments with Leishmania mexicana amazonensis kDNA have yielded genus, species, sub-species, and isolate specific kDNA fragments. Deletion mapping of a single, full-length L.mexicana amazonensis kDNA mini-circle revealed that several of the above specificities could be located within a single mini-circle and that there was a gradient of sequence variation extending from a highly conserved sequence to an isolate specific sequence.

C65 CONTROL OF TUBULIN GENE EXPRESSION DURING OPERATION OF AN "in vitro" CYCLE OF DIFFERENTIATION OF Trypanosoma cruzi, E. Rondinelli, R. Silva, C. Soares, R.S. Moura, J.F.O. Carvalho and F.T. de Castro, Instituto de Biofisica, U.F.R.J., 21941, Rio de Janeiro, Brasil

When metacyclic trypomastigote forms of T. cruzi (clone CL14) are treated with serum at 29ºC or 37ºC and transfered to LIT medium, these cells differentiate into amastigotes. The latter can be induced to differentiate into epimastigotes by incubation at 37ºC or to stump forms of trypomastigotes if incubated in high concentration of serum (50%) at 37ºC. The epimastigotes derived from amastigotes can be transformed to metacyclic trypomastigotes at 29ºC in M_{16} culture medium. This sequence of events covers complete cycle. The control of gene expression in T. cruzi is being studied in this cycle. Among the genes of our interest are the tubulin genes. The analysis of in vivo pulse labelled tubulin and in vitro translation of epimastigote and metacyclic trypomastigotes mRNA, as well as quantification of α and β tubulin mRNA by RNA blots hybridized to our α and β tubulin genomic DNA clone of T. cruzi, show that the control of tubulin gene expression during metacyclogenesis occurs at the level of α and β tubulin mRNAs accumulation. Similar study is being carried out during amastigotogenesis and epimastigotogenesis.

Supported by CNPq and FINEP

C66 CHARACTERIZATION AND ANALYSIS OF ELECTROPHORETIC KARYOTYPES FROM NEW WORLD SPECIES AND SUBSPECIES OF LEISHMANIA, John K. Scholler, Steven Reed, and Kenneth Stuart. Issaquah Health Research Institute, Issaquah, WA 98027

Leishmania are flagellated parasitic protozoans which inhabit the Old and New Worlds. Isolates are generally classified according to their growth characteristics, isoenzyme analysis, and the restriction enzyme patterns of their kinetoplastic DNA. We have used pulse field gel electrophoresis which resolves large DNA molecules to compare the species and subspecies of Leishmania. These studies reveal DNA sizes from 260 to over 2500 Kb. The size of each of the resolved DNA molecules was estimated using linear regression based on comparisons to ligated multimers of lambda phage DNA. The total number of DNA molecules and hence the genomic size was estimated by analysis of densitometric scans of the electrophoretic profiles. Each stock examined was estimated to contain at least 28 distinct chromosomal DNA molecules. The chromosomal location of several genes was established by hybridization of the blots with molecular probes and tentatively identifies homologous chromosomes among the strains and species. The chromosomal DNA pattern was distinct for each isolate examined. However, it was more similar among the isolates that are more closely related by other criteria. Two distinct processes appear to be responsible for the chromosomal DNA differences observed.

C67 CHARACTERIZATION OF RIBOSOMAL GENES OF BRUGIA MALAYI. Jyotsna S. Shah, Dyann F. Wirth and Willy F. Piessens. Department of Tropical Public Health, 665 Huntington Avenue, Boston, MA 02115.

The detailed structure of ribosomal DNA (rDNA) clone pBmr7 from microfilariae of the human filarial parasite Brugia malayi (B. malayi) has been examined using Southern blot analysis and S_1 mapping techniques. The results demonstrate that the clone pBmr7 contains two regions homologous to 28S rDNA and 18S rDNA. AccI-Sau3AI fragment from the spacer region between the 3' end of 28S rDNA and the 5' end of the 18S rDNA hybridizes strongly to genomic DNA of Brugia pahangi, a closely related filarial species. This fragment also hybridizes to genomic DNA of Dirofilaria immitis (D. immitis), another filarial species, but not to human or mosquito DNA. Therefore the fragment may be a useful probe in screening filarial infections in mixed parasite endemic areas.

C68 KINETOPLAST DNA MINICIRCLES OF TRYPANOSOMATIDS ENCODE FOR A PROTEIN PRODUCT.
Joseph Shlomai and Anat Zadok, The Kuvin Centre for the Study of Infectious and Tropical Diseases, The Hebrew University Hadassah Medical School, Jerusalem 91010, Israel.

The major constituent of the trypanosomal kinetoplast DNA (kDNA) network are several thousand duplex DNA minicircles whose biological function is still unknown. Expression of kDNA minicircles in the trypanosomatid Crithidia fasciculata was studied here by assaying directly for open reading frames of nucleotides and for the putative protein products encoded by such nucleotide sequences. kDNA minicircle fragments, inserted into bacterial plasmid vectors, were found to be expressed in the bacterial cell. Sera elicited in rabbits, by immunization with the translational products of kDNA in E. coli reacted specifically with Crithidia fasciculata cellular antigens. It is inferred that these long open reading frames are expressed in Crithidia cells. The nature of these sequences, their homology to sequences of nuclear DNA, and the properties of their encoded protein product were studied.

C69 IDENTIFICATION OF BRUGIA MALAYI IN VECTORS WITH A DNA PROBE, Betty K. L. Sim, Dyann F. Wirth and Willy F. Piessens, Harvard School of Public Health, Boston, MA 02115

This report describes a specific and sensitive DNA probe which can be applied to the field for the identification of Brugia malayi in vectors. A genomic DNA library produced from subperiodic B. malayi microfilariae was screened to detect clones containing DNA sequences which are highly repeated within the parasite genome. Several clones were further analyzed to identify those which hybridize specifically with B. malayi DNA but not with DNA from B. pahangi and Dirofilaria immitis. From these, clone pBm15 was selected because it hybridized with high sensitivity to B. malayi DNA as detected by autoradiography. Clone pBm15 could detect 150 pg of purified B. malayi microfilarial DNA. In the evaluation of the field application of this probe, we found that clone pBm15 reacted with all stages of eight different geographic isolates of B. malayi and crosshybridized with microfilariae of B. timori. It did not hybridize with Wuchereria bancrofti or with B. pahangi, W. kalimantani, Dirofilaria repens, Breinlia booliati or Cardiofilaria species, animal filariids that can be sympatric with B. malayi. Clone pBm15 correctly identified mosquitoes infected with 1 infective larvae of B. malayi. This specific DNA probe should be an invaluable tool to monitor control programs of Brugian filariasis.

C70 CHROMOSOME SIZE POLYMORPHISMS AND MAPPING OF TUBULIN GENE LOCI IN LEISHMANIA,
Terry W. Spithill and Nicholas Samaras, The Walter and Eliza Hall Institute of Medical Research, Melbourne. 3050. Australia.

Using an improved pulsed field gradient gel electrophoresis procedure, we have fractionated chromosomes (chr) of L. major, L. tropica, L. donovani, L. mexicana and L. enriettii and compared the molecular karyotypes and location of tubulin genes. L. major V121 contains at least 23 chr bands of size 250 — >2000 kb some of which are present in non-equimolar amounts suggesting there are more than 23 chr. The karyotypes of various species are very different and chr. size polymorphisms are found even among clones and strains of L. major. Genomic Southern analysis showed that there are four complete β tubulin isogenes in L. major. Mapping of individual tubulin isogenes revealed that the β tubulin repeat cluster is located on one or two large chr bands, that two dispersed β tubulin isogenes exist on chr 13 and 7, and that the isogene on chr 7 is amplified in the genome. The α tubulin cluster is located on chr 9. Comparison of tubulin gene arrangement in different species showed that α tubulin was conserved in location in four species but not in L. enriettii. All species possess a β tubulin isogene on large chr and 1-3 dispersed genes but the isogene on chr 7 is rearranged in L. mexicana and L. enriettii. The results show that L. major may be aneuploid and contain polysomic chromosomes. The differences in the chromosomal arrangement of α and β tubulin genes may be important in determining the mode of control of tubulin gene expression in various Leishmania species.

C71 MOLECULAR CLONING OF THE GENE ENCODING HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE FROM PLASMODIUM FALCIPARUM, Mark A. Sullivan, David Lloyd, Louis E. Holland, Southern Research Institute, Birmingham, AL 35255.

Parasitic protozoans lack the ability to synthesize purines de novo and therefore must salvage purine bases from host tissues to satisfy their purine requirements. Experiments with Plasmodium sp. indicate that salvage of hypoxanthine is essentially the only significant source of purines, suggesting that inhibitors of parasitic hypoxanthine guanine phosphoribosyltransferase (HGPRTase) might be useful chemotherapeutic agents. To provide sufficient quantities of the enzyme for detailed biochemical and crystallographic analysis, we have attempted to clone the gene encoding the P. falciparum HGPRTase and express it in E. coli. Using a mouse HGPRTase cDNA clone as a hybridization probe, we detected specific hybridizing fragments of P. falciparum DNA in Southern blots hybridized at low stringency. We then isolated from a mung bean nuclease-generated genomic library (McCutchan et al., Science 225:625-628, 1984) a phage containing a 675 bp insert which specifically hybridizes to the mouse HGPRTase cDNA. DNA sequence analysis of this fragment revealed an open reading frame that was 50% homologous to amino acids 30-219 of the mouse enzyme. The 5' end of the Plasmodium fragment was lacking sequences homologous to the first 29 amino acids of the mouse enzyme; instead, it contained 24 nucleotides ending with a TAG termination codon in frame with the homologous open reading frame. The sequence of this region is a very good match with the mRNA splice acceptor consensus, suggesting that mung bean nuclease cleaved within an intervening sequence to generate this clone. Further sequence analysis of genomic and cDNA clones of this region are in progress to substantiate this interpretation and to construct derivatives of the gene that can be expressed in bacteria.

C72 TRANSCRIPTION OF A REPITIVE GENE FAMILY IN TRYPANOSOMA CRUZI, John Swindle, Gregory Buck and Harvey Eisen, Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle Washington 98104.

The genomic organization and transcription of a novel repeated gene family, termed the 35-1 repeat, of T. cruzi has been studied. The 35-1 family is organized into two clusters each consisting of combinations of 700bp and 200bp repeats where the 200bp repeat represents an amplification of the 5' region of the 700bp repeat.The two clusters are seperated by approximately 4.0Kb of unique sequence. A cDNA probe for the 35-1 sequence reveales the presence of at least four stable polyA$^+$ mRNA's in northern analysis. These mRNA's range in size from 700bp to 6.5Kb. The cDNA representing the 5' most 550bp of the unit length message has been sequenced revealing the presence of a mini-exon at the 5' terminus indicating that at least one copy of the 700bp repeat represents the 5' end of the mRNA's containing the sequence. We are presently studying the organization of the 35-1 repeats within the various messages revealed by northern analysis as well as attempting to determine the relationship of the mRNA's to one another.

C73 A TANDEMLY REPEATED GENE CLUSTER OF MALARIAL PARASITES, Akhil B. Vaidya, Prema Arasu, Kathleen Suplick, and Pramila R. Anné, Hahnemann University, Philadelphia, PA 19102.

We have found that all species of malarial parasites tested (Plasmodium yoelii, P. berghei, P. chabaudi, P. falciparum, P. knowlesi, and P. cynomolgi) contain a 5.8 kb sequence that is directly and tandemly repeated in their genomes. About 150 copies of this sequence in P. yoelii make up almost 3% of the parasite genome. A molecular clone containing the 5.8 kb sequence isolated from P. yoelii hybridizes to the tandem arrays of its homologue in all of the above species of malarial parasites under stringent hybridization conditions. No hybridizable sequences are detectable with this probe in yeast, T. brucei, or mammalian DNA even under nonstringent conditions, suggesting that these sequences may be unique to malarial parasites. Pulse-field gradient gel electrophoresis of P. falciparum DNA revealed the presence of the tandem array of the 5.8 kb sequence to be restricted to those chromosomes that do not enter the gel. In both P. yoelii and P. falciparum blood stages, poly A$^+$ RNA molecules ranging from <300 nucleotides to 1.6 kb can be detected by Northern blots hybridization with the 5.8 kb probe from P. yoelii. Probing the Northern blots with RNA synthesized from the SP6 promoter indicated that the transcription of all RNA molecules proceeds in the same direction. The extraordinary conservation of this gene cluster, its expression, and its apparent amplification all indicate an important biological function for these sequences.

C74 GENETICS OF PLASMODIUM FALCIPARUM. David Walliker, Richard Carter, Isabella A. Quakyi, Thomas E. Wellems, Thomas F. McCutchan, Ana Szarfmann. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, Maryland 20205, and Naval Medical Research Institute, Bethesda, Maryland 20814.

A cross has been made between two cloned lines of Plasmodium falciparum. The lines differed in drug-sensitivity, antigens and enzyme markers, and in chromosome size as revealed by pulse field electrophoresis. Mosquitoes were fed on a mixture of gametocytes of each line, and resulting sporozoites used to infect chimpanzees. Cultured blood forms derived from these animals showed the presence of recombinants for each character examined. Chromosomes differing in size from those of each parent line were detected among the progeny, showing that extensive rearrangements occur in the genome following cross-fertilization between strains.

C75 CHARACTERIZATION AND SEQUENCE OF A HISTIDINE RICH PROTEIN OF PLASMODIUM FALCIPARUM, Thomas E. Wellems and Russell J. Howard, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD 20205.

Plasmodium falciparum synthesizes several proteins which are unusually rich in the amino acid histidine. One of these proteins (PfHRP-I) is expressed by P. falciparum isolates that induce "knobs" on infected red blood cells, but it is not produced by parasites lacking knobs. Another histidine rich protein (PfHRP-II) is produced independently of erythrocyte knobs. We have characterized properties of this second protein and have determined its corresponding nucleotide sequence from genomic clones from the P. falciparum isolate 7G8. PfHRP-II is synthesized throughout the asexual cycle and is released as a soluble protein from intact cells into culture supernatants. It is produced in all isolates of P. falciparum we have studied to date (including isolates from Southeast Asia, South America and Africa) regardless of knob phenotype or whether the parasites are culture adapted or obtained from infected hosts. The sequence data show a coding region consisting almost entirely of alanine, histidine and aspartic acid arranged in a pattern of tandemly repeating oligopeptides.

Northern and Southern blotting experiments have also shown that another gene, closely related to PfHRP-II, is expressed in infected erythrocytes. This gene is under investigation.

C76 SPECIES-SPECIFIC DNA PROBES FOR DETECTION AND IDENTIFICATION OF FILARIAL PARASITES, Steven A. Williams*+, Susan M. DeSimone* and Larry A. McReynolds+, *Smith College, Northampton, MA 01063 and +New England Biolabs, Inc., Beverly, MA 01915.

A 320 base pair repeated sequence was observed when DNA samples from the filarial parasites Brugia malayi and Brugia pahangi were digested with the restriction endonuclease Hha I. A 640 base pair dimer of the repeated sequence from B. malayi was cloned into the plasmid pBR322. Using dot hybridization, the copy number of the repeat in B. malayi was found to be about 30,000 per haploid genome. The 320 base pair Hha I repeated sequences are arranged in direct tandem arrays and comprise about 12% of the genome. B. pahangi has a related repeated sequence which cross-hybridizes with the cloned B. malayi Hha I repeat. Dot hybridization with the cloned repeat shows that the sequence is present in B. malayi and in B. pahangi but not in four other species of filarial parasites. The cloned repeated DNA sequence is an extremely sensitive probe for detection of Brugia in blood samples. Hybridization with the cloned repeat permits the detection of DNA isolated from a single parasite in an aliquot of blood from animals infected with B. malayi. There are differences in the restriction sites present in the repeated sequences that can be used to differentiate the two Brugia species. The B. malayi repeated DNA sequence is cleaved by Alu I and Rsa I but the B. pahangi sequence is not. Ten members of the Hha I repeat family were cloned and sequenced from each species. A comparison of the "consensus" repeat sequences derived from these data reveals 93% homology between the two species except in a short 60 bp region of the repeat with only 72% homology. Using these data, B. malayi and B. pahangi species-specific oligonucleotide sequences have been synthesized for use as hybridization probes.

C77 A CONSERVED DNA ELEMENT FAMILY ASSOCIATED WITH CHROMOSOMALLY INTERNAL TELOMERE REPEATS IN THE GERMLINE OF *Tetrahymena thermophila*. Claire Wyman, J. Michael Cherry† and Elizabeth H. Blackburn. Dept. Molecular Biol., Univ. of California, Berkeley CA 94720 †Dept. Molecular Biol., Mass. Gen. Hosp., Boston MA 02114

The germline micronucleus of *Tetrahymena thermophila* contains chromosomally internal blocks of the tandemly repeated sequence C_4A_2 (micC_4A_2) which is the telomeric sequence in the somatic macronucleus. Each of 6 cloned micC_4A_2 was found to be adjacent to a 30 bp conserved sequence which contains the recognition sequence for the infrequently cutting restriction enzyme BstX1. One clone, p17D59, had two C_4A_2 repeats with ~70 bp of immediately flanking sequence, including the 30 bp conserved sequences, in inverted repeat orientation. BstX1 digestion of micronuclear DNA released a prominent 9.7 kb fragment homologous to p17D59. An analogous blot of macronuclear DNA indicated this putative element is largely eliminated during development of the transcriptionaly active macronucleus. Consistent with this hypothesis Northern blots showed little if any RNA homologous to a Tel-1 probe. We have proposed that the 30 bp conserved sequences are the inverted repeats at the ends of a transposable element family, the Tel-1 family, and that the telomeric repeats are added to a free linear form prior to reintegration (Cherry and Blackburn, 1985. Cell, in press). This element family therefore differs from the previously described pseudogene-like element RIME of *Trypanosoma brucei* (Hasan *et al.*, 1984. Cell **37**, 333). Chromosomally internal telomeric repeats in *T. brucei* have been found at the 3' ends of VSG genes (Blackburn and Challoner, 1984. Cell, **36**, 447, Van der Ploeg et al, 1984. Cell, **36**, 459) which are thought to have moved to their present location by transposition/gene conversion (Boothroyd, 1985. Ann. Rev. Micro., in press). To investigate the mobility of Tel-1 elements we are analyzing their distribution within different strains of *Tetrahymena*.

Antigens and Immunity

C78 A SIMPLE MODEL FOR THE GENERATION OF ANTIGENIC VARIABILITY IN PARASITES, Zvia Agur, The Weizmann Institute of Science, 76100 Rehovot, Israel

In any system devoted to the treatment of information there is a trade-off between the ability to resist errors and the total information handling capacity of the system. In living organisms, there should exist such a trade-off between the ability to filter out random mutations and the phenotypic variability, which ensures persistence in periods of harsh environmental stress. Parasites vary in their level of exposure to stress exerted by the immune system of the host; hence their mechanisms of maintaining the balance between homeostasis and phenotypic variability can be of special theoretical and practical interest. The processing of genetic information in parasites has been modelled using a discrete multilevel network. Results point out at the relationships between the complexity of the parasite, the level of nonlinearity embedded in the processing of its genetic information, and the mechanisms it employs for maximizing its antigenic variability. It is suggested that, due to their low complexity, viruses are less resilient than other parasites; hence they can generate novel antigenic variants through the accumulation of small genetic changes. Being more complex, parasitic protozoa are more resilient than viruses and therefore cannot rely on progressive inflow of random mutations for the generation of novel antigens. Theoretical considerations suggest that the balance between resilience and variability is most efficiently controlled through the processors of the genomic information content. However, in the parasites that are constantly under stress the capacity of shifting the balance between homeostasis and variability is not employed, and a program for a continuous production of novel antigens should be directly built into the genome.

C79 TRANSCRIPTS ENCODED BY SEQUENCES UPSTREAM OF VARIANT SURFACE GLYCOPROTEIN GENES IN TRYPANOSOMA BRUCEI. Robert Aline Jr., John Scholler, and Kenneth Stuart. Issaquah Health Research Institute, Issaquah, WA 98027

Variant surface glycoprotein (VSG) gene expression in T. brucei is controlled at the level of transcription. Although the promoter for VSG gene expression has not been found, it is far 5′ to the coding sequence. We have examined transcripts from several IsTaR 1 VSG genes using probes corresponding to the adjacent upstream sequences (the co-transposed segments). These co-transposed segments, ranging in size from 1.2 kb to 5 kb, are transcribed along with the VSG coding sequences as large precursor RNAs. The size of the precursor RNAs appear to be related to the length of the co-transposed segment and the size of the upstream barren region. These precursor RNAs are subsequently processed into sets of smaller transcripts, including the mature VSG mRNA, whose size and number depends on the particular co-transposed segment. The relative abundances of several of the RNA processing intermediates differ among three different clones expressing the same VSG gene. It appears that different clones may process the same precursor RNAs in a different order or with different kinetics. The transcripts corresponding to the co-transposed segments are polyadenylated and are capped by the spliced leader sequence (the 35-mer). These sequences, however, do not contain long open reading frames and have no known function.

C80 ANTI-Tc-85 MONOCLONAL ANTIBODY INHIBITS THE INVASION OF *TRYPANOSOMA CRUZI IN VITRO*. M.J.M. Alves,G. Abuin and W. Colli, Dept.Biochemistry University of São Paulo, São Paulo, SP, Brazil

Monoclonal antibodies were raised against the surface of trypomastigote forms of *T. cruzi*, Y strain. The immunization agent employed were living trypomastigotes which have been previously inactivated with 8-methoxypsoralen. Although some of the monoclonal antibodies reacted against antigens shared by trypomastigote and epimastigote forms, the majority were trypomastigote-specific. The monoclonal antibodies specific for the trypomastigote forms recognized all infective stages regardless of their source, including trypomastigotes from the bloodstream of infected mice,insect feces, tissue culture and those resulting from differentiation of epimastigotes in axenic culture media. The monoclonal antibodies H1A10 and 6A2 partially prevented *T. cruzi* invasion of LLC-MK_2 cell monolayers (inhibition of 50-70%) when present in the culture medium throughout the entire infection experiment. Both antibodies recognized the same 85,000 M.W. glycoprotein (Tc-85), with a pI ranging from 6.3-7.5, specific for the trypomastigote surface which binds to WGA and probably contains N-acetyl-D-glucosamine and/or sialic acid. These antibodies are being used for the isolation of reasonable amounts of the antigen for chemical and immunological studies.

C81 Molecular cloning and characterization of antigens detected by antisera against the infective larvae of the human filarial nematode, Brugia malayi. Prema Arasu, Clotilde Carlow, Mario Philipp and Francine Perler, New England Biolabs, Beverly, MA 01915.

Antigens expressed by infective larvae (L3) of parasitic helminths are known to play a crucial role in stimulating host-immunity against infection. Several studies have further documented the importance of L3 antigens in experimental model systems of filariasis. In order to utilize recombinant DNA technology in identifying immunoprotective/immunodiagnostic filarial proteins, we have constructed a B. malayi genomic library in the lambda bacteriophage expression vector, λgt11. Recombinant clones were screened with rabbit antisera generated against B. malayi L3-stage antigens and 35 clones were identified by ^{125}I-Protein A assay. In Western blot analysis of B. malayi adult worm antigen extracts, the anti-L3 antiserum recognized several prominent bands indicating that the antiserum can identify antigens from both the adult and infective stages. We therefore will characterize expressed proteins for stage-specificity and species-specificity, and relevant antigens will be used to develop immunodiagnostic reagents. The protective capacity of specific recombinant antigens will also be investigated using a murine model system developed in our group.

C82 A 75K MEROZOITE SURFACE ANTIGEN FROM PLASMODIUM FALCIPARUM. Feroza Ardeshir, Janette Flint, and Robert T. Reese, Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Immunofluorescence experiments with extracellular merozoites of P. falciparum have shown previously that a 75K protein is located on the merozoite surface (Ardeshir et al., PNAS 82: 2518, 1985). The surface location of this protein has been confirmed by immunoelectron microscopy using monospecific sera raised against the expressed product of a cloned gene fragment. A cross-reacting antigen of identical molecular size is present in all the geographically distinct isolates of P. falciparum we have screened. Immunoprecipitation experiments of parasite proteins labeled during the different stages of the asexual blood cycle show that 75K protein is synthesized throughout the cycle. We are currently investigating the protective nature of the merozoite surface epitope expressed by an 860 base pair cDNA fragment which encodes part of the 75K protein. The DNA sequence of this fragment contains only one open reading frame, and the size of the predicted protein corresponds to the size of the expressed bacterial protein. The predicted amino acid sequence includes a repeating unit of four amino acids. Twenty-five out of 32 immune human sera tested reacted strongly with the 75K protein, which may therefore be important in providing immunity to Plasmodium falciparum.

C83 CLONING AND EXPRESSION OF E. TENELLA MEROZOITE cDNA, Mary-Helen Binger, Steven J. McAndrew, and Gene Schildknecht, Roche Research Center, Hoffmann-La Roche, Inc. Nutley, NJ

The developmental complexity of Eimeria tenella has been investigated by comparing the protein products of in vitro translation of mRNA prepared from sporulating and usporulated oocysts, sporozoites and merozoites. The proteins were immunoprecipitated with rabbit serum directed against sporozoites or against merozoites, and with immune chicken serum.

Expression libraries containing merozoite cDNAs were constructed in λgtll and screened with these same anti sera. Parasite antigens synthesized by individual positive clones were bound to nitrocellulose filters and used to select mono-specific antibody to the molecularly cloned antigen from the polyclonal anti-serum. This selected antibody was then used to used to immunoprecipitate the primary translation product programmed by merozoite mRNA in the rabbit reticulocyte lysate. The selected antibody also identified the mature forms of the protein on Western blots of merozoite antigens, in immunoprecipitations of ^{125}I-surface labelled merozoite protein, and in immunofluorescence using fixed parasites.

C84 ANALYSIS OF PROTECTIVE T-CELL MECHANISMS OF IMMUNITY, David A. Brake, William P. Weidanz, Elizabeth Blankenhorn, and Carole A. Long, Malaria Research Group, Hahnemann University, Philadelphia, PA 19102-1192.

The development of a blood-stage malaria vaccine requires identification and characterization of plasmodial antigens recognized by both humoral and cellular immune mechanisms. To explore cell-mediated immune mechanisms during malarial infection, we utilized the murine parasite Plasmodium chabaudi adami as a model system, since it has been shown that immunity to this parasite is mediated by T lymphocytes. Splenic T lymphocytes obtained from previously infected mice have been maintained in vitro using IL2 and antigenic stimulation. These IL2-dependent T lymphocytes proliferated in vitro in the presence of solubilized parasite preparations of homologous but not heterologous antigens. Antigen-specific proliferation was H-2 restricted. Moreover, these propagated cells were effective in adoptively transferring protection to both athymic nude mice and sublethally irradiated recipients. This protective response was dose-dependent and antigen-specific, and transferred T lymphocytes were able to confer protection even if administered late after infection. Cloned T-cell lines derived from these cells are being tested for their capacity to adoptively provide protection in vivo and for lymphokine production in vitro. In addition, we are examining rearrangements of T-cell receptor genes in these cells. These experiments will provide information on the mechanisms by which these cells mediate protection as well as identifying plasmodial antigens capable of inducing protective cell-mediated responses. Understanding of such protective cell-mediated mechanisms may have important consequences for prophylaxis and treatment of human malaria.

C85 MOLECULAR CLONING AND ANALYSIS OF GENES ENCODING *TOXOPLASMA GONDII* ANTIGENS, J. Lawrence Burg, Dalia Perelman and John C. Boothroyd, Stanford University, Stanford, CA 94305

We have constructed an expression library in the phage vector λgt11 using genomic DNA of the Coccidian parasite, *Toxoplasma gondii*. Immune serum from mice chronically infected with RH strain toxoplasma was used to isolate a sequence from the library with coding potential for a major parasitic antigenic determinant. Southern hybridization analysis has shown that this gene, termed TgB1, is arranged in tandem repeats of 2200 base pairs. Partial genomic DNA digests have revealed at least twelve tandem repeats, whereas quantitative hybridization experiments predict the existence of about 50 copies of TgB1 per genome. The TgB1 repetitive sequence appears unique to the toxoplasma genome; no hybridization is detectable with other genomic DNAs, including trypanosome, yeast, and human DNA. A complete genomic TgB1 repeat has been cloned and sequenced. Determination of the transcriptional and protein-coding regions are in progress. The antigenic determinant produced in the TgB1 λgt11 clone is recognized by immune serum from mouse, rabbit, and human.

We have also screened a toxoplasma cDNA expression library (also constructed using phage λgt11) for sequences which encode antigens present on the surface of the parasite. Polyclonal antiserum elicited against purified P30 and P22, two of the major surface antigens, was used to isolate cDNA sequences for each of these proteins. Using the P22 cDNA as a probe of a Northern transfer, we were able to detect a single RNA species about 1600 nucleotides in length. Isolation of the genomic DNA sequences for P30 and P22 and further characterization of these genes is currently underway.

C86 EPITOPE-LEVEL DELINEATION OF IMPORTANT FUNCTIONS IN INVASION OF ERYTHROCYTES BY *PLASMODIUM FALCIPARUM*. Gillian Bushell[1], Jacqueline Upcroft[1], Louis Schofield[1], Allan Saul[1], Juan Cooper[1], Ronald Epping[1], Mario Geysen[2], Stewart Rodder[2] and Chev Kidson[1]. 1. Queensland Institute of Medical Research, Brisbane, Australia. 4006; 2. Commonwealth Serum Laboratories, Melbourne, Australia.

A proportion of monoclonal antibodies (Mabs) raised against the erythrocytic stages of *Plasmodium falciparum* inhibit the invasion of erythrocytes by merozoites. Of 350 anti-parasite Mabs derived in this laboratory at least 20 are inhibitory and recognize several different classes of parasite antigens. More than one domain of a given antigen may give rise to inhibitory antibodies. The responsible epitopes may be linear or non-linear, highly variable or highly conserved among parasite strains, and may be within repeated or non-repeated sequence regions. Even within a short amino acid sequence repeat a functionally important domain may be in close proximity to immuno-dominant domains without demonstrated biological function. These observations have specific implications both for vaccine immunogen design and for description of the molecular events involved in the interaction between parasite and host cell during the invasion process. Molecular mapping of linear epitope sequences or synthetic mimicry of non-linear epitopes corresponding to inhibitory Mabs, together with evaluation of the degree of sequence conservation among isolates permits fairly precise delineation of the underlying structural requirements of critical antigens. Comparison with polyclonal immune serum allows assessment of the natural immunoresponsiveness to these functionally important antigenic domains.

C87 ANTIGENS OF EIMERIA CLONED AND EXPRESSED IN E.COLI. Clarke, L.E., Messer, L.I. and Wisher, M.H. Houghton Poultry Research Station, Houghton, Huntingdon, Cambs, U.K. PE17 2DA.

Avian coccidiosis, caused by infection with protozoan parasites of Eimeria spp., is of major economic importance to the poultry industry. At present, the disease is controlled by chemotherapy, but work is in progress to develop a non-infectious vaccine. Antigens that may be involved in the induction of species-specific immunity have been identified on the sporozoite and merozoite stages of E.tenella. Serum from chickens immunised by infection was used to precipitate radiolabelled polypeptides and to screen Western blots of parasite proteins. The ^{125}I-polypeptide profiles of sporozoites and merozoites were different; immune serum specifically precipitated sporozoite polypeptides of >220, 110-100, 51-46, 45-39, 30-26 and 18-14 Kd and merozoite polypeptides of 104-100, 31 and 28-26 Kd. Preabsorption of immune serum with merozoites inhibited the precipitation of radiolabelled sporozoite antigens, indicating that cross-reacting antigens are present on these two stages.

Genomic DNA has been isolated from E.tenella oocysts and libraries constructed in λgt11. Screening of the libraries has detected a number of eimeria polypeptides recognised by the polyspecific immune chicken serum. A selection of recombinants, whose protein products range from 8.5 to 67.3 Kd larger than β-galactosidase, are being evaluated as vaccine candidates.

C88 SELECTING CANDIDATE VACCINE ANTIGENS OF **PLASMODIUM FALCIPARUM**, Ross L. Coppel, Robin F. Anders, Graham V. Brown, Hans D. Stahl, Graeme Woodrow, A. Edward Bianco, Pauline E. Crewther and David J. Kemp, The Walter and Eliza Hall Institute of Medical Research, Victoria, 3050, Australia

We have identified over 400 **Escherichia coli** clones expressing **P.falciparum** antigens using cDNA libraries and human immune sera. These expressing clones were used as affinity reagents to prepare monospecific human antibodies. Such antibodies were used to extensively characterize the corresponding **P.falciparum** antigens with respect to molecular weight, stage specificity of synthesis and location. DNA sequencing and genomic mapping experiments provided information on the structure and isolate distribution of the antigens. At least 20 distinct antigens, many containing tandem repeats, were identified including mature stage antigens, some located in rhoptries, histidine rich proteins and antigens associated with the cytoskeleton of infected erythrocytes. This information allows a rational approach to selection of candidate vaccine antigens based on availability to the immune system, degree of variation etc. A number of these antigens including the Ring-Infected Erythrocyte Surface Antigen (RESA) are currently being assessed for their efficacy as candidate vaccine molecules.

C89 STUDY OF AN ESCHERICHIA COLI ELICITED AVIAN COCCIDIAL PROTEIN, H.D. Danforth,* P.C. Augustine*, R. McCandliss+, and M. Libel+, *USDA/ARS, Animal Parasitology Institute, Beltsville, MD, 20705, and +Genex Corporation, Rockville, MD, 20852

An avian coccidial protein (designated 5401) from the species Eimeria tenella was elicited in Escherichia coli. The mRNA was isolated from sporulating oocysts, cDNA was subsequently produced and then inserted into gtll phage vectors. The recombinant phages carrying DNA coding for the coccidial antigen were identified by use of immunoenzymatic techniques with immune chicken serum. Coccidial DNA was transferred to plasmids and the plasmids transformed into E. coli. The 5401 protein obtained from E. coli lysates was shown by SDS-PAGE to have a molecular weight of approximately 50 kd, and DNA sequence analysis demonstrated that the protein has repeated amino acid sequences. A subcutaneous injection of the protein into 3-4 week old chickens with Freund's complete adjuvant produced serum antibodies that detected both the 5401 protein and solublized sporozoite antigen with the Western blot technique. Birds that were immunized with the 5401 protein were partially protected against an oral challenge of E. tenella oocysts.

C90 CLONING OF PLASMODIUM VIVAX DNA USING AN EXPRESSION VECTOR. STUDY OF PARASITE ANTIGENS WITH MONOSPECIFIC ANTIBODIES IMMUNOPURIFIED FROM IMMUNE HUMAN SERA BY PLAQUE ANTIBODY SELECTION.Peter H. David°, Dyann F. Wirth°°, Denise M.Mattei°, Luiz S.Ozaki°, Francisco Garcia Pons° and Kamini N.Mendis°°°.° Departement d'Immunologie, Institut Pasteur, Paris, France.°°Department of Tropical Public Health, Harvard School of Public Health, Boston, MA, USA.°°°Department of Parasitology, Faculty of Medicine, Colombo, Sri Lanka.

P.vivax is a human malaria parasite the prevalence of wich is more important than that of P.falciparum in certain regions of the world. Due in part to the impossibility of maintaining the parasite in culture, little is known concerning the antigenic makeup of P.vivax. Using DNA extracted from parasites isolated from infected human blood, we have established a genomic library using the expression vector lambda gt 11. The library was screened with immune serum from P.vivax infected patients and hyperimmune serum obtained from rabbits immunized with purified gametes. A panel of positive clones were isolated. These clones were used to affinity purify antibodies directed against the P.vivax antigens expressed in E.coli ; nitrocellulose filters on which plaques of a recombinant phage had been blotted were incubated with hyperimmune human serum, washed and the bound antibodies were eluted. Such monospecific antibodies were used to characterize antigens in asexual stages and in gametes obtained from infected patients.

C91 IDENTIFICATION AND CHARACTERIZATION OF THE GENES OF THREE SPECIFIC POLYPEPTIDES OF TRYPANOMOSA CRUZI, Elizabeth A. Dragon, Stacey R. Sias, Ethel A. Kato and Darlene W. Hawkins, Codon, 430 Valley Drive, Brisbane, CA 94005

Chagas' disease, a major cause of morbidity and mortality throughout Central and South America, results from the infection of individuals with the protozoan parasite Trypanosoma cruzi. We are trying to identify proteins of T. cruzi which are targets of the human immune response. We have constructed both cDNA and genomic libraries from T. cruzi Peru strain epimastigote mRNA and chromosomal DNA. By hybrid-selection/translation we have identified three families of cDNA clones that encode three distinct immunoreactive polypeptides. We have isolated, sequenced, and mapped chromosomal DNA clones corresponding to these cDNA clones. At least one of these polypeptides is an antigen shared by both epimastigotes and trypomastigotes. We will discuss the implications of these findings with respect to developmental regulation within the parasite and the immune response of the (human) host.

C92 IDENTIFICATION AND CHARACTERIZATION OF THE GENE FOR A MAJOR SURFACE ANTIGEN OF EIMERIA TENELLA. James G. Files, Leland S. Paul, Irene Kuhn and Jeffrey D. Gabe. Codon, 430 Valley Drive, Brisbane, CA 94005.

E. tenella is a species of Coccidia that is responsible for severe losses to the poultry industry. A major surface antigen from E. tenella sporozoites and sporocysts has been identified, purified and sequenced. We have used this protein sequence to design oligonucleotide hybridization probes for the gene encoding the antigen. We have constructed libraries of E. tenella genomic DNA in bacteriophage vectors λgt-wes-λb and λ1059. A clone positive to all of the probes was isolated from each library and was sequenced. DNA sequence shows that these clones contains the entire gene encoding the antigen. The protein encoded by this gene has a structure consistent with that of a eucaryotic cell surface protein.

C93 STUDIES OF PROTEINS ENCODED BY THE TRYPANOSOMA BRUCEI EXPRESSION SITE ASSOCIATED GENE FAMILY, Carol P. Gibbs, Doris F. Cully and George A.M. Cross, The Rockefeller University, New York, NY 10021

The Trypanosoma brucei expression site associated gene (ESAG) family contains members that are located within the expression site upstream from the variant surface glycoprotein (VSG) gene and are coordinately expressed with the VSG (Cully, Ip and Cross. 1985. Cell. 42:173-182). In order to identify and elucidate the function of the ESAG protein, portions of both the 117 and 221 ESAGs have been expressed as fusion proteins in E. coli. The entire 117 ESAG, with the exception of the putative signal sequence, has been cloned into the pATH1 vector. Two constructs, containing 5' and 3' specific sequences of the 221 ESAG, have been obtained in the pEX vector. Antisera against these ESAG fusion proteins immunoprecipitatethe respective proteins from E. coli. Results of immunoprecipitations and western analyses of T. brucei extracts will be presented.

C94 DNA SEQUENCE ANALYSIS OF cDNA CLONES FOR THE M_r 185,000 GLYCOPROTEIN OF PLASMODIUM FALCIPARUM. Randall F. Howard, Feroza Ardeshir, and Robert T. Reese, Immunology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

A cDNA expression library has been created in pUC8 from poly $(A)^+$ mRNA from the Honduras I/CDC isolate. Nine thousand of the 30,000 clones were screened with Aotus immune serum (Ardeshir et al., PNAS 82:2518, 1985). Since no gp185-related clones were identified, 2 oligomeric DNA probes were synthesized from the 295 bp DNA sequence of the K1 isolate (Hall et al., Nature 211:379, 1984) and used to isolate clones by colony hybridization. The DNA sequence of the obtained clones (1052 bp) overlapped and displayed considerable homology to the sequences of the K1 isolate from Thailand (Hall et al.), the SGE2 isolate from Zaire, Africa (284 bp, Cheung et al., EMBO J. 4:1007, 1985), and the Wellcome isolate from West Africa (5920 bp, Holder et al., Nature 317:270, 1985). To produce antigen-expressing clones, cDNA was digested with BAL 31 nuclease and inserted into the expression vector pCQV2. The inserted DNA of 3 of the 14 antigen-expressing clones was sequenced and found to express protein from a single open reading frame. This reading frame coincided with those deduced for the three published sequences. However, the Honduras cDNA sequence, like the K1 cDNA sequence, contains a UGA codon 256 amino acids downstream from the putative initiation methionine of the Wellcome isolate.

C95 CLONING OF A GENE FROM SCHISTOSOMA MANSONI WHICH ENCODES A UNIQUE 97k PROTEIN RECOGNIZED BY VACCINATED MICE. D. Lanar, E. Pearce, S. James and A. Sher. Laboratory of Parasitic Diseases, NIH, Bethesda, MD 20892 and Departments of Medicine and Microbiology, George Washington University Medical Center, Washington DC 20037.

Mice vaccinated intradermally with soluble extracts of Schistosoma mansoni in conjunction with the bacterial adjuvant Bacille Calmette Guerin (BCG) show high levels of resistance to cercarial challenge infection. Antibodies from these mice recognize only a single parasite protein of M_r 97k by immunoblotting and immunoprecipitate an in vitro translation product with the same M_r.

mRNA from adult worms was used to make a cDNA library in the expression vector gtll. This library was screened with hyperimmune rabbit antisera made against affinity purified 97k protein. Two clones, MACII 97.1 and MACII 97.3, were successfully selected and purified from the intial screening of the unamplified library. The expression products of both of these clones react with antibodies from mice, guinea pigs and monkeys vaccinated with BCG plus S. mansoni protein extract as well as with a monoclonal antibody, 4B12, which recognizes the 97k protein. These clones are currently being employed to determine the sequence of the gene encoding the 97k protein. In addition, their expression products are being assayed for their ability to vaccinate mice against challenge infection in the intradermal BCG model.

C96 A LIVE RECOMBINANT VACCINIA VIRUS VACCINE AGAINST MALARIA. Christopher Langford*, Stirling Edwards**, Lynn Corcoran*, Peter McIntyre*, David Kemp*, Robin Anders* and Graham Mitchell*. *The Walter and Eliza Hall Institute of Medical Research, Melbourne Victoria 3030, Australia. **CSL, Parkville, Victoria 3050, Australia.

Live recombinant vaccinia viruses containing foreign viral surface antigen genes effectively immunize animals against a wide variety of viral infections. In the majority of cases the introduced protein is expressed in its normal context on the surface of the recombinant virus infected cell. We are investigating the use of recombinant vaccinia viruses to immunize animals with **Plasmodium falciparum** antigens which may be of importance in the development of host protective immune responses in humans. Unlike the viral antigens, many of the most interesting falciparum antigens are either not integral membrane proteins (e.g. the glycophorin binding protein and S-antigens) or are transported away from the parasite to membranes of the infected red cell (e.g. RESA, HRPs and the cytoadherence molecules). Early indications are that the **P.falciparum** antigens may not be processed or trafficked correctly in virus infected mammalian cells. A detailed analysis of the expression of a number of falciparum antigens in vaccinia virus will be presented. Methods will be described which have enabled us to retarget antigens to the surface of recombinant virus infected cells thus optimizing their immunogenicity. For example the normally non-immunogenic S-antigen protein which is secreted from recombinant virus infected cells becomes highly immunogenic upon the addition of a hydrophobic transmembrane sequence from a mouse membrane IgG protein thus anchoring the hybrid protein in the plasma membrane of virus infected cells.

C97 CHARACTERIZATION OF EIMERIA SPOROZOITE SURFACE ANTIGENS WITH MONOCLONAL ANTIBODIES, S. McAndrew, T. Truitt, G. Schildknecht, M.H. Binger, M. Griffin, and R. Chizzonite, Roche Research Center, Hoffmann-La Roche, Inc., Nutley, N.J.

Monoclonal antibodies (mAb) specific for internal and surface antigens of Eimeria sporozoites have been developed for the purpose of characterizing the immunodominant molecules which are responsible for protective immunity in the chicken. The antigens recognized by 53 mAbs have been analyzed by Western blot techniques (WB) and immunoprecipitation of sporozoite surface proteins labelled with ^{125}I. The topographical location of the antigens has been determined by indirect immunofluoresence assays (IFA) on air-dried sporozoites. By Western Blot analysis, the mAbs labelled either antigens with unique M.W.s of 6, 24, 37, 120 and 150 Kd or multiple antigens ranging from 24 to 150 Kd. The eleven mAbs which react with the 120 Kd antigen will immunoprecipitate single proteins of 105, 110 or 120 Kd. These proteins are either evenly distributed throughout the sporozoite surface or concentrated in punctate areas on the surface. The apical tip of the sporozoite is also intensely labelled with two of these antibodies. The 6 Kd and 37 Kd proteins are also concentrated on the sporozoite surface, where the 6 Kd antigen is very abundant and the 37 Kd antigen is relatively rare. Most of the MAbs which bind multiple antigens in the WB assay with E. tenella sporozoites, also crossreact with the same antigens from E. acervulina sporozoites and E. tenella merozoites. The IFA with these mAbs highlights specific labelling on the sporozoite surface. The 53 mAbs are being tested in an in vitro neutralization assay in order to determine which surface antigens are important for infection and subsequent development of the sporozoite in chicken kidney epithelial cells.

C98 Expression of a primate malarial antigen in E. coli. Marta Meda, Francine Perler, Michael Dalton, Boquin Qiang, New England Biolabs, Beverly, Massachusetts 01915.

We report the cloning of a primate malarial antigen which cross-reacts with the human malaria, P.falciparum. We previously identified a cDNA clone (pPK54), which hybrid-selects a P.knowlesi schizont mRNA encoding a 110KD protein. A genomic clone from an EMBL4 partial Sau3a library was isolated using pPK54. The genomic clone was cut separately with 6 different restriction enzymes, and then subcloned into the expression vector, Lambda gt11. Lambda gt11 allows expression of eukaryotic proteins fused to beta-galactosidase. Clones expressing the pPK54 antigen were identified by probing with Rhesus anti-P.knowlesi hyperimmune sera. Fourteen positive clones were examined, eight which expressed fused proteins and six which were expressed independently of the beta-galactosidase promoter as nonfused proteins. One nonfused protein of approximately 105KD competed out the immune precipitation of the 110KD in vitro translated antigen. A fused protein containing 50KD of the P.knowlesi antigen was isolated and used to raise immune sera in mice. The mouse sera precipitated both fused and nonfused recombinant proteins. In immune fluorescence studies, the anti-PK110 mouse sera recognized late schizonts of P.knowlesi and two geographical isolates of P.falciparum. In addition, western blot analysis confirmed that gambian sera against P.falciparum reacted with the cloned PK110 antigen.

C99 IMMUNE MODULATION OF PARASITE TRANSMISSION IN PLASMODIUM VIVAX MALARIA. Kamini N.Mendis°, Preethi V.Udagama°, Richard Carter°° and Peter H.David°°°. ° Department of Parasitology, Faculty of Medicine, Colombo, Sri Lanka. °° Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD, USA. °°°Unite d'Immunoparasitologie, Institut Pasteur, Paris.

We have demonstrated that the development of the human malarial parasite, P.vivax, can be totally suppressed in the mosquito by antibodies raised in rabbits immunized with purified gametes and by monoclonal antibodies which react with the surface of female gametes. Such anti gamete transmission-blocking antibodies are also evoked in man by natural malaria infections and occur in the sera of acute vivax malaria patients. Of 50 patients investigated in this study, two thirds had antibodies in their sera that blocked transmission of their infection by more than 95 %. Blocking immunity is readily acquired, is boosted by subsequent malaria attacks and blocking antibodies remain in the serum for about 4 months after cure. In contrast to blocking immunity, we have shown that the same immune sera that neutralised gametes and suppressed infectivity exhibited, at sub neutralising concentrations, a distinct "Infectivity Enhancing" property. The potential targets of transmission-blocking immunity were defined by studying the gamete surface components that could be radioiodinated and by determining those recognised by immune serum that blocked transmission.

C100 THE STRUCTURE OF SOME ANTIGENS OF THE ASEXUAL BLOOD STAGE OF PLASMODIUM FALCIPARUM RELATED TO THE HUMORAL IMMUNE RESPONSE

Odile MERCEREAU-PUIJALON, Denise MATTEI, Luiz OZAKI and Luiz PEREIRA da SILVA. Unité de Parasitologie Expérimentale - Institut Pasteur - 25 rue du Docteur Roux, 75724 PARIS CEDEX 15.

Genomic sequences coding for P. falciparum antigens of the asexual blood stage have been cloned in the expression vector gtll. Clones expressing parasite antigens were identified probing with different types of mono and polyspecific human, rabbit and mouse sera. DNA probes obtained from some selected clones allowed the localization of the corresponding sequences in large DNA inserts from genomic DNA library in the EMBL4 phage vector. Partial nucleotide sequence of these DNA sequences shows: 1) frequent occurrence of repetitive sequences ; 2) different levels of homology at the DNA level not obligatory corresponding to cross reactivity at the serological level.

Immunisation of mice, rabbits and squirrel monkeys was performed with either the fusion recombinant molecules or with synthetic peptides corresponding to the cloned DNA sequences. Immunological analysis shows significant functional of the antibodies raised by one or other type of molecules as well as those raised by drug-controlled infection of squirrel monkeys or natural infections in man.

C101 AN IMMUNOGENIC ANTIGEN OF THE NONLETHAL MURINE MALARIAL PARASITE PLASMODIUM YOELII ASSOCIATES WITH CLASS I MHC GENE PRODUCTS, Rona J. Mogil, Douglas R. Green and Curt Curtis L. Patton, University of Alberta, Edmonton, Alberta T6G 2H7 and Yale University, New Haven, CT 06510

Reticulocytes infected with the nonlethal variant of Plasmodium yoelii 17X (PY17X-NL) express elevated levels of Class I, but not Class II, major histocompatibility complex (MHC) antigens when compared with nonparasitized reticulocytes. In contrast, Class I antigens are not detectable on erythrocytes parasitized by the lethal variant PY17X-L. In addition, the responder status of various strains of inbred mice positively correlates with the levels of Class I MHC antigens expressed on PY17X-NL parasitized red blood cells (PRBC). MHC antigens are known to restrict or guide immune responses. However, earlier studies have failed to demonstrate H-2 restricted activity in the effector arm of immunity to blood-stage murine malaria. Therefore, we have examined the induction of immunity by irradiated PY17X-NL PRBC. No MHC restriction was observed in the ability of PRBC to immunize recipients. However, employing PRBC bearing low, intermediate or high levels of Class I antigens, we found that the levels, rather than the haplotype, of Class I antigens expressed on irradiated PRBC greatly influenced their ability to induce immunity. Furthermore, Class I-associated antigens were isolated as an immunogenic complex with anti-Class I MHC antigen antibody. Such complexes induced immunity in vivo, suggesting a biologically important mechanism by which nonlethal, reticulocytic form of malarial parasites may immunize their hosts.

C102 GENOMIC AND CHROMOSOMAL ORGANIZATION OF THE TRYPANOSOMA BRUCEI ISTAR 1 EARLY AND LATER VATs. Peter Myler, Robert Aline, Jr., Janette Allison, John Scholler, and Kenneth Stuart. Issaquah Health Research Institute, Issaquah, WA 98027.

The genomic environments of the gene family members for six early and later VATs of the IsTaR 1 serodeme of T. brucei (IsTats 1.A, 1.1, 1.3, 1.5, 1.7, and 1.11) have been determined from cDNA and genomic clones and Southern mapping using genomic DNA from these six original VATs, and two series of relapses from these VATs. The chromosomal locations of these gene copies have been determined using pulse-field gradient electrophoresis using genomic DNA from these six original VATs, and two series of relapses from these VATs. The IsTaR 1 serodeme has four megabase-sized chromosomes (1.5 - 3.0 Mb) in addition to one smaller (375 kb) chromosome, numerous minichromosomes (50 - 150 kb) and an unidentified number of large chromosomes which do not migrate into the agarose gel. We have identified VSG genes on three minichromosomes, the four megabase-sized chromosomes and the larger chromosome(s). By comparing the restriction maps of the "sub-telomeric" regions 5′ to the barren region upstream of several expressed and unexpressed VSG genes, we have demonstrated that IsTaR 1 serodeme trypanosomes are capable of expressing VSG genes in at least 8 telomeric sites, all on the four megabase-sized chromosomes. Since the VATs we have studied in our serodeme have been largely derived from single relapses from cloned populations, we have been able to explain the switches in VSG gene expression in terms of two mechanisms: gene replacement and telomeric activation.

C103 IDENTIFICATION OF AN ENTAMOEBA HISTOLYTICA ADHESINE USING ADHESION-DEFICIENT MUTANTS AND MONOCLONAL ANTIBODIES, Esther Orozco, Mario A. Rodríguez, and Rossana Arroyo. CINVESTAV IPN, Apdo Postal 14-740, México 07000, D.F.

Entamoeba histolytica adhesion-deficient mutants were isolated from clone C_9 (strain HM1:IMSS) by chemical mutagenesis. Mutants were selected by killing highly phagocytic trophozoites by exposure to bacteria labelled with bromodeoxiuridine and irradiation with 310 nm light. From ten phagocytosis-deficient mutants, isolated by this way, three were adhesion-deficient. Meanwhile, monoclonal antibodies (Mab) were prepared against the wild type strain, clone C_9. Mab against the surface of the wild type strain were selected by indirect immunoflourescence. Three Mab did recognize the wild type strain surface, but did not stain the adhesion-deficient mutants. These were tested as inhibitors of adhesion, phagocytosis and cytopathic effect, two of them inhibited the three functions. Amebic proteins from the wild type strain and from the adhesion-deficient mutants were transfered to nitrocellulose filters and incubated with the two inhibitor Mabs, both recognized a protein of approximately 110 Kd in the wild type strain that was diminished or absent in the three adhesion-deficient mutants.

C104 THE STRUCTURE OF SOME ANTIGENS OF THE ASEXUAL BLOOD STAGE OF PLASMODIUM FALCIPARUM RELATED TO THE HUMORAL IMMUNE RESPONSE, Odile Mercereau-Puijalon, Denise Mattei, Luiz Ozaki and Luiz Pereira da Silva, Unité de Parasitologie Expérimentale, Institut Pasteur, 25, rue du Docteur Roux, 75724 Paris Cedex 13.

Genomic sequences coding for P. falciparum antigens of the asexual blood stage have been cloned in the expression vector lambda gt11. Clones expressing parasite antigens were identified by screening with different types of mono and polyspecific human, rabbit and mouse sera. DNA probes obtained from some selected clones allowed the localization of the corresponding sequences in large DNA inserts from a genomic DNA library in the lambda EMBL4 vector. Partial nucleotide sequence of these DNA fragments shows:
1) frequent occurence of repetitive sequences ; 2) some degree of homology between several gene products.

Immunisation of mice, rabbits and squirrel monkeys was performed either with the fusion recombinant molecules or with synthetic peptides corresponding to the cloned sequences. The antibodies raised to these molecules display different characteristics. These differ significantly from those antibodies raised by infection in squirrel monkeys or man.

C105 Cloning of the Gene Encoding a Major Surface Polypeptide of Treponema pallidum That Reacts Strongly with Sera from Syphilis Patients. Kenneth M. Peterson, Joel B. Baseman, and John F. Alderete. University of Texas Health Science Center, San Antonio Texas 78284.

A limitation to progress in vaccine and immunodiagnostic test development for syphilis remains the inability to grow Treponema pallidum in vitro. Attempts were undertaken to utilize recombinant DNA technology to clone treponemal genes coding for proteins with important biological properties. A genomic library consisting of 10-20 kilobase partial digest fragments of T. pallidum DNA was constructed in bacteriophage lambda. Proteins of T. pallidum expressed by the recombinant phage were identified with sera from experimentally infected rabbits. Six treponemal proteins detected in recombinant phage plaques were characterized further by sodium dodecylsulfate polyacrylamide gel electrophoresis and blotting of Escherichia coli lysate proteins onto nitrocellulose. One phage clone contained T. pallidum DNA coding for a T. pallidum protein reactive with a monoclonal antibody directed against a highly immunogenic surface protein of this parasite (Infect Immun 42:623-627). All sera from patients with the different stages (primary, secondary and latent) of syphilis possessed antibody to this protein antigen. Purification of this antigen from E. coli extracts has been performed in order to examine its structure-function properties. These studies should contribute further to our understanding of the biology of this sexually-transmitted disease agent and assist in defining syphilis pathogenesis.

C106 CHARACTERIZATION AND cDNA CLONING OF A PROTECTIVE ANTIGEN FROM TOXOPLASMA GONDII. Jeffrey B. Prince, Jack S. Remington and Somesh D. Sharma, Research Institute, Palo Alto Medical Foundation, and Stanford University School of Medicine, Stanford, CA 94305.

The Toxoplasma gondii (TG) antigen recognized by monoclonal antibody (mab) F_3G_3 has been shown to protect mice against a lethal challenge of TG given one week after immunization (J. Immunol. 133:2818, 1984). In the current work we demonstrated that immunization with this antigen provides remarkably prolonged protection. Mice immunized with affinity purified F_3G_3-antigen and challenged six months later survived (>9 months), whereas all control mice died. The protection was shown to be specific for TG, since immunization with F_3G_3-antigen did not protect against a lethal challenge with Listeria.

To clone the genes encoding TG antigens, we have constructed a tachyzoite cDNA library in the expression vector λgt11. Initial screening of the library was performed with polyclonal mouse antibody against F_3G_3-antigen. Western blot analysis verified that this polyclonal antibody recognized a single protein identical in size (apparent MW of 58,000) to the antigen recognized by mab F_3G_3. Recombinant clones isolated by screening with the monospecific antibody probe contained a TG DNA insert of about 250 bp, which represents a coding capacity for one sixth of the intact protein. Preliminary results of dot blot analysis, using bacterial lysates prepared from recombinant lysogens, suggest that the recombinant antigen from these clones is also recognized by mab F_3G_3. Efforts are currently under way to isolate the entire F_3G_3-antigen coding sequence by screening the cDNA library with a nucleic acid probe derived from the 250 bp insert.

C107 The Formation of Hybrid VSG Genes in Trypaosoma equiperdum. C. Roth, G. Thon, T. Baltz and H. Eisen. Dept. of Immunoparasitology, Pasteur Institute, 75015 Paris, France.

The variable surface glycoproteins (VSGs) of T. equiperdum are expressed in a loosely reproducible order. We have been interested in determining the molecular mechanism that defines the order in which the VSG genes are expressed. Previous studies have indicated that the BC gene of a VSG expressed early during an infection completely represents the gene in the ELC. In contrast, the ELC for VSGs expressed later are hybrid genes. We have examined several of the hybrid genes in more detail to determine how the are created. We find that in the case of VSGs-78 and-20 the 3' end of the ELC is donated by a BC gene that has very little, if any, homology with the 5' BC gene. Yet, when the ELC for VSG-78 was created on two different occassions, the same hybrid 3' end was created. Additionally, the BC gene used for the 5' region of the VSG-78 gene is an incomplete gene lacking a normal 3' end. The data suggest that the same gene was in the expression site just before the VSG-78 gene on both occasions and the 5' BC gene recombined with it in the same manner two times. We propose that the formation of hybrid genes in this manner is related to the ordering of VSG gene expression.

C108 A high MW antigen associated with the red cell membrane of cells infected with Plasmodium falciparum trophozoites and schizonts. Allan Saul, Jeff Lyons and Russell Howard, Laboratory of Parasitic Diseases, NIH, and Department of Immunology, Walter Reid Army Institute of Research.

A number of new antigens appear to be associated with the membrane of red cells infected with malaria parasites. In addition to the RESA antigen present in ring infected cells, at least three antigenically distinct proteins are associated with the red cell membrane in cells infected with more mature parasites. These are a histidine rich protein; a high MW protein exposed on the surface which may be the protein responsible for mediating the cytoadherance of infected cells; and another high MW protein which is not exposed on the surface of infected cells as judged by its ability to be surface labelled or react with antisera in intact cells.

The latter protein is expressed by both knob positive and knob negative cells, but the mechanism resulting in the association of this protein with the red cell membrane appears to be altered in knob negative cells. A portion of a gene coding for an antigenic determinant recognized by monoclonal antibodies directed against th s protein has been cloned. The fragment codes for a peptide containing an 11 amino acid repeat.

C109 THE CLONING AND EXPRESSION OF LEISHMANIA DONOVANI ANTIGEN GENES, Haynes W. Sheppard, Naval Biosciences Laboratory, Oakland, CA 94625, and Dennis M. Dwyer, Laboratory of Parasitic Disease, N.I.H. Bethesda MD, 20205

Genomic and cDNA libraries of Leishmania donovani gene segments have been constructed in the bateriophage expression vector, λgt11. A large group of clones which express L. donovani antigens have been isolated using various polyvalent antisera. Groups of clones have been identified which have different patterns of crossreactivity with these antisera. Many of the clones appear to encode parasite membrane antigens some of which are recognized by sera from patients with visceral leishmaniasis. Some clones reacted with subsets of kala azar sera while others reacted with all kala azar sera tested. In addition, some of the clones appear to encode conserved antigens that are recognized by sera from mice immunized with L. major. Preliminary characterization of several clones inticated that each one contains a distinct genetic element and that they encode different fusion peptides.

C110 MURINE MALARIA DECREASES HEMOPOIETIC STEM CELLS AND TOTAL BONE MARROW CELLULARITY, Paul H. Silverman, John C. Schooley and Lynn J. Mahlmann, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

Studies on cellular changes in bone marrow during infection with murine malaria reveal a depletion of hemopoietic stem cells (CFU-s) during the later stages of infection of Plasmodium berghei and P. vinckei. CFU-s are the pluripotent cells responsible for the production of all erythroid, granulocytic and megakaryocytic cell lineages as well as the immune T & B lymphocytes. The depleted or impaired CFU-s population might account for, or substantially contribute to, the major syndromes of anemia and immunosuppression associated with malaria. These results offer an explanation of why stem cell stimulators of bacterial origin enhance nonspecific resistance to hematazoan parasites and suggest new strategies for prophylactic and treatment procedures.

C111 CHARACTERIZATION OF GENOMIC STRUCTURES AND TRANSCRIPTIONAL UNITS OF METACYCLIC VSG GENES IN TRYPANOSOMA B. RHODESIENSE. Hyeung-Jin Son, Michael J. Lenardo, Klaus M. Esser, Lekkala V. Reddy and John E. Donelson, Dept. of Biochemistry, University of Iowa, Iowa City, IA 52242 and Dept. of Immunology, Division of Communicable Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20012

Metacyclic trypanosomes in tsetse fly salivary glands repeatedly express a limited subset of 10 - 15 variable antigen types (M-VATs) that are independent of the bloodstream variable antigen types (B-VAT) orginally ingested by the fly. This suggests that specific mechanisms exist which activate the M-VAT genes, and prevent expression of the B-VAT genes, at the metacyclic developmental stage. To search for potential developmental regulation signals of metacyclic VSG genes, we have cloned, mapped and sequenced the 5'- and 3'-flanking regions of the telomere-linked VSG genes expressed in M-VAT 4 and M-VAT 7. The 5'-flanking regions do not possess a "barren region" of 76 bp repeats as do many bloodstream VSG genes but do have different kinds of repeats and an ESAG-like sequence (Cully et. al. Cell 42:173-182 (1985)). The 3'-downstream regions also possess conserved blocks of sequences. Discrete transcription units of these VSG genes have been identified by nuclear transcription experiments.

C112 HYBRID FORMATION BETWEEN AFRICAN TRYPANOSOMES DURING CYCLICAL TRANSMISSION
J.Sternberg[1], A.Tait[1], L.Jenni[2], S.Marti[2], J.Shweizer[2], B.Betschart[2], R.W.F.Le Page[3] J.M.Wells[3], P. Paindavoine[4], E.Pays[4] and M.Steinert[4]; [1]Dept.of Genetics, Edinburgh University,Scotland; [2]Swiss Tropical Institute,Basel,Switzerland; [3]Dept.of Pathology, University of Cambridge, England; [4]Dept.of Molecular Biology, Free University of Brussels, Belgium.

Two clones of T. brucei trypanosomes have been mixed and used to infect tsetse flies. After full cyclical development of the trypanosomes in the flies, hybrid trypanosomes have been identified by electrophoretic analysis of a range of enzymes and with DNA probes for single copy genes and surface antigen genes. These results demonstrate that genetic exchange takes place in trypanosomes.

The genetics of this system is now being investigated by (i) The analysis of kDNA inheritance, where initial data suggests uniparental inheritance in hybrids. (ii) Chromosome analysis on pulse field gels.

C113 ESTABLISHMENT OF AN IN VITRO TRANSCRIPTION SYSTEM IN TRYPANOSOMES,
Richard Sutton and John Boothroyd, Stanford University, Palo Alto, CA.

Synthesis of mature mRNAs in African Salivarian trypanosomes occurs by a discontinuous process. A small RNA of 137 nucleotides (medRNA) is transcribed from a DNA repeat of 1.35kb, and the first 35 nucleotides of the medRNA end up at the 5' end of most trypanosome mRNAs. As a first step in dissecting the molecular mechanisms of this process, we have established an in vitro transcription system which accurately transcribes the 1.35kb DNA repeat. Cell-free extracts were prepared from bloodstream trypanosomes. Transcription reactions were initiated by addition of extract, salts, cations, cloned 1.35kb template DNA, and radioactive UTP. Labelled RNA products were fractionated on denaturing acrylamide gels. Maximal accurate transcription was observed at 50ug/ml template DNA, 50mM KCl, and 5mM of both $MgCl_2$ and $MnCl_2$. Transcription was inhibited by 50% at a concentration of alpha-amanitin of 10ug/ml. In order to gain greater insight into the regulation of medRNA synthesis, we mapped the DNA sequences necessary for specific in vitro transcription. No sequences 3' of the transcription initiation site were necessary, whereas sequences approximately 70 nucleotides upstream of the start-site were required for accurate transcription. Comparison of these results with data from nuclear runoff experiments in trypanosomes and knowledge of the three RNA polymerases of higher eukaryotes suggest that trypanosomes have a fourth type of RNA polymerase which transcribes the 1.35kb DNA repeat which encodes medRNA.

C114 **EXPRESSION OF TRYPANOSOME CALMODULIN GENES**
Christian Tschudi, Elisabetta Ullu, Donald M. Crothers and Frank F. Richards
Yale MacArthur Center, New Haven, CT 06510

Calmodulin, an intracellular calcium-binding protein, mediates the activity of a large number of enzymatic reactions and biochemical pathways. We have shown that the calmodulin genes in Trypanosoma brucei gambiense are arranged in three identical tandemly repeated units. Several transcripts of similar size originate from these genes. A 35-nucleotide spliced leader sequence is present at the 5'-end of each mRNA but is not encoded contiguous to these genes. We are studying the transcription and processing of the calmodulin genes both in vitro, using a homologous cell-free system, and in vivo.

C115 ISOLATION AND CHARACTERIZATION OF cDNA AND GENOMIC CLONES ENCODING LEISHMANIA DONOVANI PROMASTIGOTE SURFACE ANTIGENS, Anne E. Wallis and W. Robert McMaster, University of British Columbia, Vancouver, B.C. V6T 1W5, T.W. Pearson and R.W. Olafson, University of Victoria, Victoria, B.C.

In order to characterize the major cell surface proteins on L. donovani promastigotes, two types of DNA libraries were constructed: cDNA libraries in the expression vector λgt11 and genomic libraries in the vector λEMBL3. These libraries are currently being screened by immunological and DNA hybridization techniques. A panel of monoclonal and polyclonal antibodies against promastigotes, isolated promastigote membranes and purified promastigote cell surface proteins are being used to identify specific cDNA clones which code for cell surface antigenic determinants. Concurrently, synthetic oligodeoxyribonucleotides corresponding to unique amino acid sequences of the 60,000 D major promastigote surface glycoprotein are being used to isolate specific cDNA and genomic clones.

C116 MALARIAL ANTIGEN IN THE ERYTHROCYTE MEMBRANES OF P. CHABAUDI-PARASITIZED RED BLOOD CELLS. Chingchai Wanidworanun, John W. Barnwell, Aoi Masuda and Hannah L. Shear, New York University Medical Center, New York, NY 10016.

Recently , significant attention has been paid to the existence of malarial antigens expressed on the membranes of parasitized erythrocytes and their possible role in protective immunity. In this study, we have purified erythrocyte membranes from P. chabaudi-infected erythrocytes by affinity chromatography. Mice immunized with the purified membranes produced antibodies that bind specifically to the membranes of glutaraldehyde-fixed or methanol-fixed P. chabaudi-parasitized erythrocytes. The binding gave a membranous fluorescence pattern on the membranes of parasitized erythrocytes containing the ring, trophozoite or schizont stages. The proteins recognized by immune serum migrate as a doublet in SDS-PAGE and have M.W.'s of 100 adn 110 kD, which we have designated Pc100 and Pc110. In order to further characterize and determine the role of these antigens in P. chabaudi malarial immunity, we have produced monoclonal antibodies of the same specificity. One of the monoclonal antibodies, 8F2G9, has the isotype γ_1, K and gave positive immunofluorescence at 6 ng/ml of purified protein. The monoclonal antibodies are being utilized to purify the Pc100 and Pc110 proteins for immunization studies and to determine the role of humoral immunity to these antigens. In addition, the biosynthesis of the antigen will be determined by immunoprecipitation of metabolically labeled and surface radioiodinated proteins of parasitized erythrocytes.

C117 ISOLATION OF SALMONELLA TYPHIMURIUM ANTIGENS FROM A λ GENOMIC LIBRARY WHICH INDUCE A PROLIFERATIVE RESPONSE IN PROTECTIVE, SALMONELLA-SPECIFIC T LYMPHOCYTES, Richard L. Warren and Karen A. Shalala, Wright State University, Dayton, OH 45435

Several studies have indicated that subcellular material isolated from Salmonella typhimurium provides protection against lethal challenge and is capable of eliciting delayed-type hypersensitivity (DTH). However, the nature of the antigens responsible for T lymphocyte activation is still unclear. We have previously reported that T cells raised against spent medium antigens (SMA) can provide significant, long term protection when adoptively transferred to hypersusceptible, naive mice. Antiserum raised against SMA was used to screen a lambda genomic library of S. typhimurium. Phage expressing SMA antigens which reacted with the anti-SMA antiserum were isolated and purified. Antigen preparations from the lambda clones were tested for their ability to stimulate salmonella SMA-specific T cells. Two of the four antigens tested induced a significant proliferative response, whereas antigen prepared from the lambda vector alone was not stimulatory. Salmonella antigen expressed by one of the positive clones was identified by infecting E. coli "maxi" cells. This lambda clone expressed a 54K protein that was immunoprecipitated by anti-SMA antibody. The 54K protein was not immunoprecipitated from "maxi" cells infected with lambda wes. Since the T cell populations are protective, they provide an in vitro screen of the cloned antigens which may be involved in host protection.

C118 BLOOD STAGE ANTIGEN GENES OF PLASMODIUM FALCIPARUM, James L. Weber and Jeffrey A. Lyon, Walter Reed Army Institute of Research, Washington, DC 20307-5100

Various antibody preparations ranging from total immune serum to monoclonal antibodies were used to select clones encoding malaria blood stage antigens from a genomic DNA expression library prepared using DNA from the CAMP strain of P. falciparum. Genes coding for antigens with molecular weights from 45,000 to about 300,000 were selected. cDNA probes reverse-transcribed from mRNA isolated from synchronized parasites were used to identify the stages at which the antigen genes are transcribed, and these results were confirmed by hybridizing the cloned DNA back to the RNA. Nick-translated total parasite DNA was used to identify genes containing repetitive sequences. To date, two genes have been characterized in detail, one encoding a variable merozoite surface glycoprotein of 195,000 molecular weight (gp195), and the other encoding a secreted antigen with molecular weight of 126,000 (p126) (Delplace et al (1985) Molec. Biochem. Parasitol., in press). The sequence of the gp195 gene from the CAMP strain, like the other published sequences for this gene, showed tandem repeats of three amino acids, but the CAMP repeats of Ser Gly Thr and Ser Ala Gln differed from the repeats of the other strains. Sequence comparisons as well as DNA hybridization experiments also revealed large differences in the 3' portions of the genes. Analysis of the expression proteins produced by clones containing different overlapping portions of the gp195 gene allowed mapping of the epitopes recognized by several monoclonal antibodies and formulation of a processing scheme for this antigen. The sequence of a segment of the p126 gene also contained short tandem repeats.

C119

Babesiosis vaccine: the presence of a B. bovis protective antigen in B. bigemina

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A small protective polypeptide was previously identified in Babesia bovis by immuno blotting and IFA techniques using two distinct monoclonal antibodies. An antigen of similar molecular size has now been demonstrated in B. bigemina by immuno blotting with the same monoclonal antibodies. The IFA staining patterns with monoclonal antibodies against this polypeptide are similar in both parasites.

In challenge experiments cattle immune to B. bigemina were as strongly protected against virulent B. bovis infection, as were cattle immunised against B. bovis either by live organisms or by crude parasite extracts. A fraction containing the small polypeptide derived from B. bigemina was used to immunise two groups of splenctomised calves, one of which was subsequently challenged with B. bigemina the other with B. bovis. The results of this challenge are discussed.

C120 ANTIBODIES AGAINST ISOLATED *TRYPANOSOMA CRUZI* ANTIGENS INHIBIT PARASITE INTERIORIZATION INTO MAMMALIAN CELLS, Bianca Zingales, Monika V. de Arruda, Walter Colli. Dept. Biochemistry, University of São Paulo, Brazil

Trypomastigote surface antigens are involved in *T. cruzi* interiorization into mammalian cells. Polyclonal antibodies were raised in rabbits against some *T. cruzi* (Y strain) antigens isolated by preparative SDS-PAGE. The IgG fraction against either the 90-80 K or the 60-50 K polypeptides of tissue culture trypomastigotes (TCT) inhibits parasite interiorization into LLC-MK_2 cells. A 70% inhibition and a 50% inhibition is observed, respectively, for TCT (Y strain) and for axenic culture trypomastigotes (ACT) (CL strain). A correlation exists between the concentration of immune IgG and the extent of inhibition. In contrast, antibodies obtained against polypeptides of equivalent M.W., isolated from epimastigotes (non infective forms), show a less pronounced inhibitory effect (up to 25%). The surface antigens of TCT and ACT were identified after radioiodination of living parasites and immunoprecipitation with the antisera. Analyses by 1D and 2D gel electrophoresis suggest the presence of common antigens in the two strains and of shared epitopes among the antigens. These antigens could be involved in a general mechanism of parasite interiorization. The protective effect of these antisera against experimental Chagas' infection is being tested.

Biochemistry

C121 USE OF HYBRIDOMA ANTIBODIES IN THE STUDY OF HOST CELL-PARASITE INTERACTION. P.C. Augustine and H. D. Danforth, USDA/ARS, Animal Parasitology Institute, Beltsville, Maryland, 20705

Hybridoma antibodies (Hab) have been developed against Eimeria sporozoites and against cultured host cells that the parasites invade. A number of Hab that react with surface or surface/internal antigens of E. tenella sporozoites inhibit the invasion of cells by these parasites. These sporozoite Hab recognize antigens of different molecular weights (MW) ranging from 5-8 kd to greater than 90 kd. The Hab causing the greatest inhibition of invasion had a MW of 22 kd and appeared to recognize an antigen that was an integral part of the parasite surface membrane. Other sporozoite Hab with MW of 5-8 kd caused less inhibition of invasion; the antigens recognized by these Hab appeared to be transiently associated with the sporozoite surface membrane. Hybridoma antibodies produced against host cell surface membrane antigens also showed varying abilities to inhibit invasion of cells by Eimeria sporozoites. One cell surface Hab recognized soluble proteins with MW of greater than 100 kd; interaction of this Hab with antigens on the host cell surface had little effect on invasion by sporozoites. Another Hab recognized host cell membrane proteins of 37 and 45 kd, and was capable of reducing invasion by 40%. Collectively, the data show that Hab interaction with either the Eimeria sporozoite or host cell surface membrane may interfere with the invasion process.

C122 POST-TRANSLATIONAL PROCESSING AND TRANSPORT OF A TRYPANOSOMA BRUCEI VARIANT SURFACE GLYCOPROTEIN, James D. Bangs, Norma W. Andrews, Gerald W. Hart and Paul T. Englund, Johns Hopkins School of Medicine, Baltimore, MD 21205

The variant surface glycoprotein (VSG) of T. brucei undergoes several co-translational modifications: removal of an N-terminal signal sequence, addition of N-linked oligosaccharides and replacement of a C-terminal peptide with a glycolipid membrane anchor. Using the variant ILTat-1.3, we have analyzed, quantitatively, the kinetics of 3 processes that occur subsequent to these initial events: 1) conversion of 56 and 58 Kd polypeptides to mature 59 Kd VSG, 2) transport to the cell surface, 3) transport to a site where VSG is susceptible to membrane-bound phospholipase C. Cells were pulsed 2 min with [^{35}S]methionine, chased and these processes were analyzed by: 1) mobility of VSG polypeptides in SDS-PAGE, 2) accessibility of labeled VSG to membrane-impermeable crosslinker, 3) susceptibility of labeled VSG to release by hypotonic lysis. The $t_{1/2}$ of all three processes is 12-15 min. Tunicamycin and monensin have no effect on the kinetics of processes 2 or 3. A 54 Kd species is detected in the presence of tunicamycin and this species increases slightly in apparent M_r during the chase period, suggesting that novel processing event(s), perhaps on the glycolipid, may occur. Monensin prevents conversion of 58 Kd VSG to 59 Kd VSG, suggesting that this event(s) may occur in the Golgi. The comparable kinetics of these three processes suggest that transport of VSG from the site of maturation to the plasma membrane is rapid.

C123 Secretion of Acid Phosphatase by Leishmania donovani, Paul A. Bates and Dennis M. Dwyer, Lab of Parasitic Diseases, NIAID, NIH, Bethesda, MD 20892.

Promastigotes and amastigotes of L.donovani produce and release an extracellular soluble acid phosphatase (AcP). Monoclonal antibodies and a monospecific polyvalent rabbit antiserum were produced against the AcP. These sera were used to follow the synthesis and release of this glycoprotein from promastigotes in pulse-chase metabolic labelling experiments. Cumulative results demonstrated that the AcP is rapidly synthesised and secreted into the culture medium within 20 minutes. Tunicamycin inhibited N-linked glycosylation and reduced the apparent molecular weights of both intracellular and extracellular forms of the enzyme on SDS-PAGE, but the rate of secretion was unaffected. These results constitute the first report of a secretory pathway in a parasitic protozoan. Further, the energy invested in the rapid synthesis and release of the AcP suggests that it may play a major role in establishment and/or maintenance of the host-parasite relationship.

C124

HMG-CoA REDUCTASE: ROLE IN SCHISTOSOME EGG PRODUCTION, J.L. Bennett and E. Vandewaa, Michigan State University, E. Lansing, MI 48824

HMG-CoA reductase plays an important role in regulating the synthesis of cholesterol, polyisoprenoids, terpenes and ubiquinones. This enzyme catalyzes the conversion of HMG-CoA to mevalonate. Mevinolin (MV) is a selective and potent inhibitor of this enzyme that is used to lower cholesterol levels in mammals. When MV was administered daily (250 mg/kg) to mice infected with S. mansoni it selectively inhibited production of eggs by the parasite and the gross pathology (enlarged liver and spleen) associated with this infection. MV (10^{-5} M) also inhibited egg production by schistsomes kept in culture, but this could be reversed by adding mevalonate (20 µM) to the culture medium. Metabolism of C-14 acetate into polyisoprenoids, by cultured schistosomes, is inhibited by MV (10^{-5}). This inhibition is associated with a significant reduction in the availability of mannose-lipid intermediates that are responsible for the glycosylation of schistosome proteins. Lastly, schistosomes exposed to low doses (50 mg/kg) of MV, while in their host, will produce six times more eggs when removed from their host and placed in a culture medium free of MV. These results suggest that inhibition of schistsome HMG-CoA reductase reduces the concentration of nonsterol lipid vital for the production of eggs and that the synthesis of this enzyme, similar to the mammalian enzyme, is markedly stimulated when exposed to inhibitors.

C125

SPECIFIC IDENTIFICATION OF INFECTIVE LARVAE OF BRUGIA MALAYI IN MOSQUITOES USING AN ANTI-SURFACE MONOCLONAL ANTIBODY, Clotilde K.S. Carlow and Mario Philipp, Molecular Parasitology Group, New England Biolabs, 32 Tozer Rd, Beverly MA 01915

The ability to identify third stage filarial larvae (L3) in insect vectors is crucial in epidemiological studies, and in the assesment of filariasis control measures. However, different genera and species of such larvae, which are sympatric in many regions, cannot be distinguished easily, either morphologically or histologically. An IgM monoclonal antibody has been produced which reacts with L3 of the human filaria B. malayi and not with L3 of many other species, including the closely related Brugia pahangi and Wuchereria bancrofti, or with different developmental stages within the species B. malayi. The target epitope is on the surface of the larvae, as revealed by immunofluorescence (IFAT). Both dead and live parasites displayed equal intensity of fluorescence, and were detectable within Aedes aegypti mosquitoes. An alternative, more practical assay has been developed for identifying larvae during field studies. Worms were incubated sequentially with: a) Monoclonal Ab, b) A biotinylated anti-mouse Ig antibody, c) Horseradish peroxidase linked to streptavidin, d) 4-chloro-naphtol (substrate). B. malayi L3 were stained blue, whereas L3 of the closely related species B. pahangi remained unstained. This test can be performed easily, rapidly and individual larvae identified precisely. Attempts to identify the surface antigen bearing the species-specific epitope are in progress.

C126

Schistosoma mansoni Adult Worm Proteinase: Tissue Localization in the Parasite and Antibody Response in Infected Animals. Cynthia L. Chappell and Marc H. Dresden. Baylor College of Medicine, Houston, TX 77030.

A cysteinyl proteinase, SMw32-P, from the digestive tract of Schistosoma mansoni adults has been purified to homogeneity (single band on silver-stained SDS-PAGE) by centrifugation, molecular sieve chromatography (AcA54) and immunoaffinity chromatography. This proteinase has an apparent molecular weight of 32,000 daltons, is inhibited by E-64 and does not appear to be glycosylated. A monoclonal antibody (Mc 110) to the proteinase was generated from the spleen cells of an infected mouse. Mc 110 has an IgG1 (K chain) isotype and binds to Protein A. This antibody has been used in histochemical studies (LM and EM) to localize the proteinase to the worm gut. SMw32-P is immunogenic in mice, baboons and humans. Kinetic analysis of the antibody response in mice has shown a transient IgM increase along with sustained high levels of anti-SMw32-P IgG and IgE. Preliminary studies with sera from S. mansoni-infected baboons and humans have revealed an anti-SMw32-P response that is not seen in the normal control sera. These results suggest that the SMw-32P proteinase is a potent immunogen in schistosome infection and may be useful as a marker of disease.

C127 IN VITRO EFFECTS OF TOXIC PRODUCTS OF LIPID PEROXIDATION ON PLASMODIUM FALCIPARUM.
G.A. Butcher, G.D. Buffinton, W.B. Cowden, N.H. Hunt and I.A. Clark. Australian National University, Canberra, Australia.

Evidence is accumulating that free radical-induced lipid peroxidation could be important in cell-mediated killing of malaria parasites. Using HPLC, we have found that exposure of P. vinckei-infected red cells to oxidative stress generates high concentrations of n-alkanals and 2-alkanones, and significant amounts of 4-OH-2-alkenals and 2,4 alkadienals. Smaller, though significant, amounts of most of these compounds are generated in parasitized red cells that are from heavily parasitized mice and have not been exposed to exogenous oxidative stress in vitro.

We have tested these aldehydic products of lipid peroxidation for their effects on the growth of the erythrocytic stage of P. falciparum. At various concentrations (10-100 μM), n-alkanals (C6-C11), 2-alkenals (C4-C9), 2-alkanones (C3-C9) and malonyldialdehyde had no effect on [^{3}H] hypoxanthine uptake. In contrast, the 2,4-alkadienals (C7, C9, C10) and 4-OH-2-alkenals (C6, C8, C9) inhibited incorporation by up to 80% at 50 μM and 99% at 100 μM. We have measured these concentrations in P. vinckei-infected red cells exposed to 1 mM H_2O_2 or t-butyl hydroperoxide, and demonstrated by light microscopy that the parasites are, in each case, inhibited intraerythrocytically. The formation of these products from lipids exposed to H_2O_2 released by phagocytes could contribute to both suppression of the parasite during cell-mediated immunity and the tissue damage found in malaria.

C128 STRUCTURAL AND IMMUNOCHEMICAL ANALYSIS OF TRYPANOSOME VARIANT SURFACE GLYCOPROTEINS
Michael W. Clarke*[1], Tony F. Barbet+, Robert W. Olafson* and Terry W. Pearson*, Univ. of Victoria*, Victoria, Canada and Wash. State Univ., Pullman, Wa., U.S.A.

Two variant surface glycoproteins (VSGs) with shared surface epitopes from T. brucei clones WaTat 1.1 and 1.12, have been compared biochemically. Attempts were made to identify and localize VSG-specific epitopes (both surface exposed and cryptic) on the purified VSGs. The two VSGs have identical molecular weights, different PI's, similar amino acid compositions and highly shared N-terminal amino acid sequences. High-performance liquid chromatographic (HPLC) analysis of cyanogen bromide and trypsin fragments revealed both shared and unique peptides. That the secondary structure of the two VSGs (along with other unrelated VSGs) is conserved was suggested by the similar retention times of both soluble and membrane forms of VSGs on reserse-phase HPLC columns. Monoclonal antibodies directed against surface epitopes failed to react with peptides or with VSGs which had been denatured. Conversely, antisera to VSG peptides failed to react with the surface of living trypanosomes. These data indicate that the VSG epitopes exposed on the surface of living trypanosomes are assembled topographical sites dependent on folding of the polypeptide. In order to determine which peptides comprised such epitopes an ^{125}I-labelled heterobifunctional crosslinking-transfer reagent was used. Evidence was found for the involvement of several peptides in composition of the surface epitopes whereas non-surface epitopes involved only a single peptide indicative of a segmental site. Taken together these studies suggest a highly convoluted configuration for surface-exposed VSG epitopes. [1]Current address: Univ. of Western Ontario, London, Canada.

C129 RNA POLYMERASES IN TRYPANOSOMA BRUCEI
Albert W.C.A. Cornelissen, Frans Brekelmans, Jan M. Kooter and Piet Borst, The Netherlands Cancer Institute(Antoni van Leeuwenhoekhuis), Amsterdam, The Netherlands.

The RNA polymerases of T.brucei have been characterized in an in vitro system, in which elongation of nascent RNA in a run-on assay with isolated nuclei(1) was used in the absence or presence of different α-amanitin concentrations (2,3). The unexpected outcome of these experiments was that the RNA polymerase transcribing the Variant Surface Glycoprotein genes is atypical with respect to the inhibition by α-amanitin (2,3). Transcription of the genes coding for rRNA, tubulin and 5S RNA is respectively, insensitive, sensitive and moderately sensitive to α-amanitin as would be expected for transcription of these genes in an eukaryote (4). Transcription of VSG genes, however, is completely insensitive to α-amanitin, even at concentrations up to 1000 times higher than is required for RNA polymerase II in other systems (4). To investigate the genes coding for RNA polymerases in T.brucei, we have isolated genomic clones using recombinant clones of Drosophila (5) and yeast (6), encoding the large subunit genes of polymerase I, II and III. We are currently charaterizing the genomic clones of T.brucei.

(1) Kooter, J.M. et al. (1984) EMBO J. 3, 2387-92 ; (2) Kooter, J.M. and Borst, P. (1984) Nucl. Acids Res. 12, 9457-72 ; Laird, P.W. et al. (1985) Nucl. Acids Res. 13, 4253-66 ;(4) Roeder, R.G. (1976) in: RNA Polymerases pp. 285-329 Cold Spring Harbor Laboratory Press ; Greenleaf, A.L. et al. (1983) J. Biol. Chem. 258, 13403-6 ; Sentenac, A. unpublished results

C130 ISOLATION OF cDNA CLONES AND DIFFERENTIAL EXPRESSION OF mRNA ENCODING THE S. MASONI HEMOGLOBINASE, Alan H. Davis, Jayasri Nanduri, Ronald Blanton and Adel A. F. Mahmoud. Case Western Reserve University, Cleveland, Ohio 44106

One of the few schistosome proteins whose function is known is the hemoglobinase utilized for degradation of host hemoglobin. To characterize expression of gene(s) encoding this enzyme we have isolated cDNA clones from an expression library constructed in λgtll amp3. The 27 kd hemoglobinase was purified from adult schistomes by gel filtration and affinity chromotography as described by Sauer and Senft (Comp. Biochem. Phys. 42B: 205, 1972). The purified protein was used to immunize rabbits. Western blot experiments indicated the resulting antiserum was specific for hemoglobinase. The antiserum was then used to immunologically screen the expression cDNA library. Five recombinants were isolated which specified production of hemoglobinase epitopes either fused or unfused to β-galactosidase. The cDNA insert from one clone, approximately 370 bp long, was used to probe Northern blots of poly (A^+) mRNA isolated from various stages of the S. masoni life cycle. Hybridization to a 1400 base mRNA was only observed to adult worm mRNA but not to egg or cercaria RNA. Hybridization to RNA isolated from separated male and female worms indicated the hemoglobinase RNA was present in higher abundance in females. Full length cDNA clones are being isolated for DNA sequence analysis.

C131 THE ROLE OF HEAT SHOCK PROTEINS IN DIFFERENTIATION OF TRYPANOSOMA BRUCEI. Charles E. Davis, D.G. Guiney and M.E.M. Colmerauer, UCSD, School of Medicine, San Diego, CA 92103

Near the peak of parasitemia in the mammal, the Trypanosoma brucei group differentiates from rapidly-replicating long-slender forms to short-stumpies that do not replicate in the mammal but are necessary to infect the tsetse. We have shown previously that indomethacin accelerates differentiation, while theophylline blocks the process, in association with changes in intratrypanosomal cAMP.

In an attempt to find natural regulators and markers of differentiation, we probed lysates of long-slenders, short-stumpies and procyclics (insect mid-gut form) for heat shock proteins (hsp), because trypanosomes experience several temperature changes during their life cycle. Anti-chicken hsp-70 (from M. Schlesinger, Wash. Univ., St. Louis, MO.) detected prominent 70K dalton bands in immunoblots from SDS-PAGE of each morphologic form. Furthermore, novobiocin, which blocks the heat shock response of Drosophila, also blocks differentiation of T. brucei in mice. In 3 separate experiments, T. brucei in novobiocin-treated mice never differentiated and the parasitemia never dropped below 2×10^7, while control mice experienced 2 cycles of parasitemia.

These results raise the possibility that differentiation of T. brucei, which moderates parasitemia and is essential to the life cycle, may be triggered by hsp that are induced by the febrile response of infected mammals.

C132 METHOTREXATE RESISTANCE IN CRITHIDIA FASCICULATA IS DUE TO DECREASED UPTAKE OF DRUG, Homero Dewes, Hanne L. Ostergaard and Larry Simpson, Biology Department and Molecular Biology Institute, University of California, Los Angeles, CA 90024

The trypanosomatid, Crithidia fasciculata, developed resistance to methotrexate (MTX), an inhibitor of dihydrofolate reductase (DHFR), when challenged with low drug concentrations (2-5 μM). During the period of adaptation cell division was arrested and the cells showed abnormal morphology, but cell death did not occur. Reversion to normal morphology and resumption of cell division occurred after 80 hr of drug treatment. The adapted cells survived at high levels of drug (1-2 mM) and the resistant phenotype was stable in the absence of the drug. Several independently derived lines of resistant cells showed neither gene amplification nor altered DHRF in terms of activity or MTX sensitivity. Instead, resistance was correlated with impaired uptake of [^{3}H]-MTX, which in wild-type cells is transported by a carrier-mediated, energy-dependent process.

C133 THE MECHANISM OF THE PHOTODYNAMIC ACTION OF CRYSTAL VIOLET ON THE BLOODSTREAM FORM OF Trypanosoma cruzi. Roberto Docampo[1], Ronald P. Mason[1], Fernando S. Cruz[2], and Silvia N.J. Moreno[1], [1]Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA, and [2]Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Transmission of Chagas' disease by transfusion of blood containing Trypanosoma cruzi has often been reported, and crystal violet is widely used by blood banks in attempts to eliminate such transmission. Previous studies (Docampo et al. Science 220, 1292, 1983) have demonstrated a photodynamic action of crystal violet on T. cruzi epimastigotes. When whole human blood containing crystal violet was irradiated with visible light, the ESR spectrum of the ascorbyl radical was detected. Similar results were also observed when blood from mice containing T. cruzi trypomastigotes was used. Spin-trapping studies using 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) revealed the formation of superoxide anion upon illumination of blood containing crystal violet (i.e. a superoxide dismutase-sensitive, catalase-insensitive DMPO-hydroxyl adduct signal). Similar results were observed in incubations containing ascorbate, crystal violet, and DMPO in the absence of blood. Hydrogen peroxide generation was also detected in these incubations, thus confirming redox cycling of crystal violet under aerobic conditions. Irradiation of T. cruzi-infected blood containing crystal violet significantly enhanced the killing effect of crystal violet on T. cruzi. The addition of ascorbic acid to these incubations further enhanced the effect of crystal violet and fully protected inoculated mice from infection.

C134 3'-NUCLEOTIDASE OF LEISHMANIA DONOVANI - EVIDENCE FOR EXONUCLEASE ACTIVITY, Michael Gottlieb and Gary W. Zlotnick, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205

A membrane bound 3'-nucleotidase has previously been identified at the surface of the human pathogen Leishmania donovani, and other trypanosomatid protozoa. In addition to its ability to hydrolyze 3'-ribonucleotides, the enzyme also functions as a nuclease as indicated by the following: i) the nucleotidase and nuclease activities copurify during enzyme isolation and ii) nucleotidase and nuclease activities comigrate in nondenaturing polyacrylamide gels as well as in SDS gels. The substrate preference for the nuclease is poly U > poly A >> poly C > poly G. The products of nuclease digestion are 5'-mononucleotides, which can serve as substrate for a surface membrane bound 5'-nucleotidase. The cumulative data suggest that the exonuclease/3'-nucleotidase, in concert with the ecto-5'-nucleotidase, plays an important role in the initiation of purine salvage from nucleotides and nucleic acids in L. donovani which is incapable of de novo purine synthesis. (This work was supported by grants to Michael Gottlieb from the National Institutes of Health (AI-16530) and UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases).

C135 IDENTIFICATION OF A PARASITE PROTEIN IN THE MEMBRANE OF PLASMODIUM FALCIPARUM - INFECTED ERYTHROCYTES. Kasturi Haldar and George A. M. Cross, Rockefeller University, New York, NY 10021.

Schizont erythrocyte membranes of P. falciparum Gambian clones FCR-3/A2 and FCR-3/D4 isolated on Affigel 731 beads, contain a parasite protein of 102-kD. The protein is synthesized at the schizont stage and cannot be detected earlier than 36 h in the life cycle. It is present in both K+ (A2) and K- (D4) clones. The intensity of the coomassie stained protein band is comparable to that of spectrin, indicating that it is abundant in the infected cell. Preliminary experiments indicate that the protein is not heavily glycosylated. The presence of covalently bound lipid and phosphate is being investigated

C136 ISOLATION AND CHARACTERIZATION OF A NEURAMINIDASE, A DEVELOPMENTALLY REGULATED ANTIGEN OF Trypanosoma cruzi. Guenter Harth, Constantine G. Haidaris, and Magdalene So. Research Institute of Scripps Clinic, La Jolla, CA. 92037.

The parasitic protozoan T. cruzi, the causative agent of Chagas' disease, undergoes a series of differentiation stages during its life cycle. Upon conversion from the insect or epimastigote form, which is non-infectious, to the pathogenic or trypomastigote form the parasites show a number of morphological and physiological changes which are reflected by a differential expression of stage specific antigens. These help the parasites to escape the immune response of the host and to survive intracellularly.

Following the initial observations of Pereira (1) we set out to purify and characterize the neuraminidase of T. cruzi. The enzyme activity is developmentally regulated and greatly stimulated in trypomastigote forms. Non-infectious and intracellular forms exhibit less than 10% the activity of trypomastigote forms. Cell fractionation studies show that the enzyme is a membrane bound antigen and can be shed into culture supernatants. We purified the enzyme about 1000 fold from both sources through several chromatography steps to 60% homogeneity as revealed by SDS-PAGE analysis. A single band of M_r 60,000 is detected in Western blots using Chagasic sera as a probe. The neuraminidase is a glycoprotein, the molecular weight of which drops to 52,000 after endoglycosidase F treatment. Epimastigote forms that were converted into metacyclic trypomastigote forms in vitro also show an increase in neuraminidase activity comparable to tissue culture trypomastigotes. Current studies aim at the cloning of the corresponding gene and elucidating the role the enzyme plays during the process of infection.

(1) Pereira, M.E.A. 1983. Science 219: 1444 - 1446.

C137 LEISHMANIA AMASTIGOTES INCORPORATE AND TRANSFORM MACROPHAGE HOST CELL STEROLS, George G. Holz, Jr.[a], L. John Goad[b], Leige Galvao-Quintao[c], Jan S. Keithley[c], and David H. Beach[a], [a]S.U.N.Y. Health Science Center at Syracuse, Syracuse, NY 13210, [b]University of Liverpool, Liverpool, L69 3BX, United Kingdom, [c]Cornell University Medical College, New York, NY 10021.

Leishmania braziliensis guyanensis [MHOM/SR/80/CUMC 1, clone 1-A] promastigotes grown at 25°C in RE III medium supplemented with hemin, peptone, lysed whole rabbit blood and 10% heat-inactivated fetal calf serum contained as major sterols [identified by GC/MS] cholesterol (50%) from the culture medium and endogenous fungal-type sterols; ergosterol [assuming a 24ß-methyl] (20%), ergosta-5,7,24(28)-trien-3ß-ol [5-dehydroepisterol] (15%), ergosta-7,24(28)-dien-3ß-ol [episterol] (5%). Amastigotes grown in and isolated from the murine macrophage tumor cell line J774.1(N^-) by the methods of Colomer-Gould et al. [J. Exp. Med. 162, 902-916 (1985)] contained host cell cholesterol (55%) and cholesta-5,24-dien-3ß-ol [desmosterol] (10%), and a variety of Δ^5-sterols with structures suggesting their origin by parasite metabolism of the desmosterol; ie., cholesta-5,24-dien-3ß-ol → ergosta-5,24(28)-dien-3ß-ol [24-methylenecholesterol] (10%) → ergosta-5-en-3ß-ol [24-methylcholesterol] (∿1%) → ergosta-5,22-dien-3ß-ol (∿1%). The proportion of each of the two major endogenous sterols seen in promastigotes was sharply reduced [ergosterol (2%), 5-dehydroepisterol (1%)], while that of episterol (6%) was unchanged. Other amastigote stage-specific sterols were ergosta-7-en-3ß-ol (∿1%) and stigmasta-7,24(28)-dien-3ß-ol (4%), probably arising, respectively, by reduction and by methylation of episterol.

C138 GLYCOCONJUGATES OF LEISHMANIA IDENTIFIED BY LECTIN-BLOTTING, Charles L. Jaffe and Diane McMahon-Pratt, Biophysics, Weizmann Inst. of Science Rehovot, Israel and Yale Univ. School Med., New Haven, CT. 06510

Lectin-blotting was used to identify the different carbohydrate containing components in promastigotes of Leishmania. Several stocks were examined: L.d.infantum, L.m.mexicana, L.m.amazonensis, L.b.braziliensis, L.b.panamensis, L.enriettii, L.major and L.tropica. All the parasites studied contained glycoconjugates which reacted with concanavalin A and lentil lectin. Seven of nine stocks showed multiple bands with molecular weights(Mr) from 27 to >200 KD. Overall staining patterns between Leishmania exhibited little similarity, however a doublet(Mr=41-52KD) and a triplet(Mr=160, 175 and 185KD) were present in eight stocks. The reactivity of concanavalin A and lentil lectin were very similar. Binding patterns obtained with ricinus communis-120, soybean and peanut agglutinin were relatively simple. Peanut agglutinin only reacted with L.major(Mr=35, 49 & 52KD), L.enriettii(Mr=35 & 52KD) and L.b.panamensis(Mr=52KD). An additional component migrating with the dye front was also identifited. R.communis reacted with every stocked except for L.tropica. Six stocks showed diffuse bands, Mr=14-60KD and/or Mr=150-240KD. Soybean agglutinin weakly bound to all the Leishmania examined. Each stock contained a different set and number of bands(Mr=26, 30, 34, 46, 48 and 50KD). No bands were observed for wheat germ agglutinin. Changes were found by lectin-blotting with peanut agglutinin, lentil lectin and concanavalin A in anattenuated L.major stock passaged in vitro for over one year.

C139 PURIFICATION OF PLASMODIUM FALCIPARUM PROTEIN WHICH ACTIVATES NON MALARIA SENSITIZED HUMAN T-LYMPHOCYTES : G. Jaureguiberry (*), W. Ogunkolade (§), J.J. Ballet (=), A. Rhodes-Feuilette (§), B. Andrieux (+) and M. Agrapart(=) : (*) Lab. Hématologie Expérimentale ; (§) Lab. Immunomodulation and (=) Unité INSERM 108, Lab. Immunologie : Hôp. St-Louis, 2 Pl. Dr. Fournier,75476 Paris Cédex 10, France. - (+) Institut de Médecine et d'Epidémiologie Tropicale, Fondation Léon M'Ba, Hôp. Claude Bernard, 10 Av. de la Porte d'Aubervilliers, 75019 Paris, France.

A mitogenic activity (m.a.) for human T-lymphocytes from malaria non sensitized donors is detectable in Plasmodium falciparum culture supernatants. In studies done with synchronized P. falciparum cultures, m.a. was found at the end of the schizonte stage. The activity was associated with an induction of interferon (IFN-γ) and interleukin 2. Activated T-lymphocytes exhibited both helper and suppressor/cytotoxic phenotypes/functions and several long terme T-cell lines/clones were obtained.

These results led us to initiate an in vitro study in order to answer the following question : can one, isolate, from P. falciparum parasitized human red blood cell supernatants (PF-RBCS) substances with the capacity to induce IFN-γ and with a T-mitogenic activity. By gel filtration and SDS-PAGE analysis a 70 Kd protein with a mitogenic activity was characterized in P. falciparum cultures supernatants.

Using labelling experiments in vitro, it was shown that the protein was synthesized by the parasite. Also, the capacity of the protein to induce IFN-γ has been demonstrated. Other immuno-interactions of the protein are currently investigated.

C140 PLASMODIUM FALCIPARUM-DERIVED TRANSFERRIN RECEPTORS ON THE SURFACE OF INFECTED ERYTHROCYTES. Michele Jungery and Mario H. Rodriguez-Lopez, Harvard School of Public Health, Boston, MA 02115

Iron is essential for malaria parasite metabolism. Although it has been assumed that plasmodia obtain iron from haemoglobin, evidence now suggests that the parasite is unable to catabolize haemin and has to obtain iron from the plasma.

We have observed that, unlike control cells, parasite infected erythrocytes take up ^{59}Fe and ^{125}I-labelled transferrin in culture. Light microscopic examination of live parasitized red cells incubated with FITC-labelled transferrin showed that some of the transferrin bound to these cells, was internalized, and appeared to accumulate around the parasitophorous vacuole. The labelled transferrin/receptor complex has been separated by chromatography on Sephadex G 200 columns and the elution from these columns has been analyzed by SDS-PAGE. Further, the receptor for transferrin on the surface of P. falciparum infected erythrocytes has been separated by affinity chromatography on transferrin-sepharose columns. Analyses of co-immunoprecipitated parasite labelled proteins using antibodies directed to both transferrin and to parasite specific proteins are in progress.

C141 STRUCTURAL AND ANTIGENIC CHARACTERIZATION OF A L. MEXICANA MEMBRANE PROTEIN, Lesley P. Kahl, Diane McMahon Pratt, Harvard Medical School, Boston, MA 02115

A L. mexicana amazonensis promastigote membrane glycoprotein (Mr 46,000) containing the species-specific, promastigote-specific and host-protective epitope of monoclonal antibody IX 2H7-E10(M-2) has been purified to homogeneity and studies made to determine the minimum peptide fragment that retained antigenic activity. The M-2 epitope was readily destroyed by limited proteolysis and/or reduction and alkylation indicating disulphide bond involvement in its formation by secondary or tertiary protein structure. The stability of approximately half of the molecular mass of the protein (46kDa/M-2) was dependent upon disulphide bonds within it. Enzymic digests under various conditions resulted in a glycopolypeptide (Mr 22,000 to 27,000) which was extremely resistant to further proteolysis and which was the dominant immunogenic portion of the purified protein recognized by a specific rabbit heteroserum. No smaller or larger fragments were antigenic. Studies using the radioiodinated hydrophobic probe (3-(Trifluoromethyl)-3-(Iodophenyl Diazirine) (TID) revealed that 46kDa/M-2 was an integral membrane protein with a component polypeptide (Mr 23,000 to 27,000), highly resistant to further proteolysis and containing sequences within the lipid bilayer of the external promastigote membrane. Data indicate that the TID-labeled fragment is identical to the immunodominant fragment. We suggest that hydrophobic interactions maintain the integrity of this fragment as amino acids within it fold through the lipid bilayer. The composition and relationship to antigenicity of the carbohydrate portion of 46kDa/M-2 was determined. The single N-linked oligosaccharide chain, cleaved by endoglycosidase H was not involved in the epitope of M-2.

C142 CHARACTERIZATION OF AN IMMUNOGENIC NEUTRAL THIOL PROTEINASE FROM ENTAMOEBA HISTOLYTICA. William E. Keene, Diane J. Banda, and James H. McKerrow. School of Public Health, Univ. of California, Berkeley, and Depts. of Medicine and Pathology, Univ. of California, San Francisco, CA 94143.

Characterization of the major neutral proteinase purified from the culture supernatant (CS) of axenic HM-1 strain Entamoeba histolytica trophozoites suggests that it may play a key role in the pathogenesis of extraintestinal amebiasis. The enzyme, a thiol proteinase with subunit M_r of ~56000, degraded a model of extracellular matrix, as well as purified laminin, fibronectin, and type I collagen. Purified enzyme induced a cytopathic effect on BHK cells in culture, probably by degrading anchoring proteins such as fibronectin and laminin. Aside from directly mediating tissue invasion and destruction, the thiol proteinase is a plasminogen activator, by virtue of its ability to cleave polypeptides C-terminal to arginine. As this is also characteristic of complement proteinases, we tested the ability of CS to generate chemotaxins in human serum. Normal serum incubated with CS stimulated leukocytes to migrate 148 μm in 35 min (controls: buffer, 75 μm; zymosan, 158 μm). Incubation with heat-treated serum, however, did not stimulate migration (77 μm), indicating that CS is not a direct C5 convertase. Antibodies to the enzyme can be detected (by ELISA and Western blot) in sera from patients with amebiasis, suggesting its potential as a serodiagnostic reagent. We are currently investigating the dynamics of the antibody response to this enzyme in human patients with a spectrum of infections.

C143 UNIQUE BIOPHYSICAL PROPERTIES OF THE SURFACE OF PARASITIC NEMATODES, Malcolm W. Kennedy, Michael Foley, Yien-Ming Kuo and John R. Kusel, Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Glasgow G61 1QH, Scotland.

The epicuticle of parasitic nematodes comprises their exposed outer surface, and is thought to be a primary target for immune attack. However, many nematodes are manifestly resistant to immune elimination. The mechanism by which this is achieved is unknown, but could be due to the rapid shedding of surface antigens which has been demonstrated in several nematode parasites. It would be expected that such a surface would exhibit rapid lateral mobility of lipids and proteins when examined by biophysical techniques. Here we have investigated two persistent parasites of man : the infective larvae of Trichinella spiralis and Toxocara canis. We measured the affinity of fluorescent lipid probes for the surface lipid layer, and estimated their lateral mobility by Fluorescence Recovery after Photobleaching(FRAP). The probes were based on the fluorescein or indocarbocyanine fluorochromes, the former bearing a single aliphatic chain of 12-18 carbon, and the latter with two identical chains of 12-18 carbon. We found a selectivity for these probes which is unique to the epicuticle : of all the lipid analogues used only 5-N-(octadecanoyl)-amino-fluorescein inserted. Using this probe, FRAP measurements revealed that the surface lipid was virtually immobile. This was in contrast to that of the somulum of Schistosoma mansoni, which showed a degree of restriction, but not to the remarkable degree exhibited by the nematode epicuticle. These findings further emphasise the unique character of the epicuticle, and its lack of homology with the cellular plasma membrane.

C144 INCREASE OF SOLUBLE Fc-RECEPTORS IN MOUSE SERUM DURING SCHISTOZOMA MANSONI INFECTION. D.KHAYAT, Z.DUX, D SERBAN, D.GOLD, C.JACQUILLAT.

Molecules carrying a specific affinity for the Fc domaine of IgG can be released in vitro from different cells or cell-lines and are called immunoglobulin binding-factor (IBF) (FRIDMAN et al.1974). IBF have been shown to act, in vitro, as regulatory molecules involved in the regulation of the production of immunoglobulins by activated B-cells. We have shown that such IBF or better called cell-free Fc-Receptor (Cf-FcR) exist and circulate under a functionnal form (capable of binding Fc domaine of IgG) in normal mouse serum (KHAYAT et al.1984). The amount of Cf.FcR in serum of adult mice, which seems to be genetically determined, was shown to be undetectable in new-born mice with an increase with age (KHAYAT et al.1984) and to stay undetectable in germ-free adult recipients (PURE et al.1984).

We have individually tested the amount of Cf.FcR in the sera of 11 schistozoma mansoni infected mice and 9 normal control mice, both groups being kept in the same conditions, at day 0 (before infection) and on days 22, 41, 62 and 82 after the first control bleeding. We report the dramatic increase of the level of Cf-FcR in mouse serum carrying the parasite, with a stable level on sex, on age and strain-matched control mice. That increase seems to be parallele to the rise on the amount of IgG in the sera of infected mice. Infection seems therefore to modulate the expression of Cf-FcR in mouse serum. Such Cf-FcR, by establishing a functional relationship with circulating IgG, may modulate the humoral response of infected animals. (This work was supported on part by ARC grant N°6933).

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C145 PRIMARY STRUCTURE AND SECONDARY STRUCTURE POTENTIALS OF TWO TRYPANOSOMA CONGOLENSE VARIANT SPECIFIC GLYCOPROTEINS, T. Lalor, D. Binder, J. Strickler, J. L'Italien, G. Shimamoto, S. Wait, J. Novotny, J. Radding, W. Konigsberg, M. Armstrong and F. Richards, Yale University, New Haven, CT.

The primary structures of two variant specific glycoproteins (VSG) of the Nannomonad Trypanosoma (T.) congolense are presented. These proteins are the locus of antigenic variation. The secondary structure potentials of both VSG's have been calculated. The amino acid sequence and secondary structure potentials of these VSGs have been compared with those of several T. brucei complex VSGs. In homologous regions the T. brucei complex VSGs show a pattern of sharply contrasting secondary structure potentials. It has been suggested previously that this pattern gives rise to different folding structures in different members of this polygene protein family. Thus different short regions of the protein sequence are exposed as antigenic "caps" on the solvent exposed surface of intact trypanosomes. A sharply contrasting secondary structure potential pattern is also found in regions of the two T. congolense VSGs. However, there is no discernible homology of primary structure between each of the two T. congolense VSG's and any member of the T. brucei comlex VSG polygene family whose primary structure has been determined. One possible explanation of these results is that the evolutionary pressures of parasitism have caused two different cell surface protein families to adopt similar organisational principles to achieve antigenic variation at the phenotypic level.

C146 TAENIAESTATIN, A CESTODE PROTEINASE INHIBITOR WITH BROAD HOST REGULATORY ACTIVITY, R. Wes Leid, Christine M. Suquet, R.F. Grant and T. Yilma, Washington State University, Pullman, WA 99164-7040

Taeniaestatin, a proteinase inhibitor from larval cestodes, inhibits murine lymphocyte proliferation to PHA, CON-A, PWM and ovalbumin by >90%. This inhibition is mediated partly by inhibition of IL-2 generation, as exogenous IL-2 stimulation of murine lymphocyte proliferation is not inhibited. IL-1 stimulation of murine thymocyte proliferation is also inhibited by 100% in a dose-dependent manner at 5 units of taeniaestatin. Alternative or classical complement pathway activation was inhibited in a dose-dependent manner, with a pronounced effect on factor D of the alternative pathway. Dose-dependent inhibition of neutrophil migration to the chemotactic ligand $C5a/C5a_{des-arg}$ was also observed. This inhibition was reversible as shown by washing and restimulation with chemotactic factor. Inhibition of replication of vesicular stomatitis virus and encephalomyocarditis virus in mammalian kidney cells was seen, with >80% inhibition at doses of 2 units of taeniaestatin. Two other viruses tested, vaccinia and infectious bovine rhinotracheitis, were not inhibited at any dose of taeniaestatin tested. In addition, >85% inhibition of murine myeloma cell proliferation was observed at a dose of 2 units of taeniaestatin. Inhibition of cellular activation and function in all cases was without cellular cytotoxicity at the highest dose levels of taeniaestatin used, as assessed by exclusion of vital dyes, LDH release and uptake of fluorescein acetate.

Supported in part by NIH Grant AI-19713 to RWL.

C147 STRAIN VARIATION IN THE CIRCUMSPOROZOITE PROTEIN GENE OF PLASMODIUM FALCIPARUM, Michael J. Lockyer and Ralph T. Schwarz, Wellcome Biotechnology Ltd., Langley Court, Beckenham, Kent, U.K.

An investigation has been made into heterogeneity in the circumsporozoite protein gene of P. falciparum in one West African and four Thai strains. Southern blotting experiments using the cloned gene from the West African strain show a variation between strains in the number of 12 base pair tandem repeats and in the presence of Xho II sites within the repeats. This variation has been further analysed at the DNA sequence level.

C148 9-DEAZAINOSINE AS A CHEMOTHERAPEUTIC AGENT FOR TRYPANOSOMA CRUZI: BIOLOGICAL EFFECTS AND METABOLISM. Randolph L. Berens, Douglas L. Looker and J. Joseph Marr. University of Colorado Health Sciences Center, Denver, CO 80262

Previous studies have shown that certain inosine analogs selectively inhibit the growth of the parasite protozoan responsible for leishmaniasis, Chagas disease, and African sleeping sickness. One of these analogs, 9-deazainosine (9-DINO) was very effective against the culture forms of these pathogens. We would like to report the results of a more in depth investigation of this compound's effects and metabolism in Trypanosoma cruzi, the causative agent of Chagas' disease. Growth inhibition studies in epimastigote cultures resulted in ED_{90} values of 3 μM for the inosine analog sensitive Peru strain and 15 μM for the inosine analog resistant CL strain. When 9-DINO was added to T. cruzi infected tissue culture cells (WI-38VA-13) it was found that the Peru strain was eliminated after three subcultures in the presence of 3.7 μM while the CL strain infection was eradicated by 18.5 μM in the same number of subcultures. Metabolic studies using radioactive 9-DINO showed that both epimastigote and infected tissue culture cells convert the drug to its respective analogs of IMP, IDP and ITP as well as aminating its 9-DINO-MP to give 9-deazaadenosine mono-, di- and triphosphate. A similar incubation with uninfected tissue culture cells resulted in only the formation of the 9-DINO nucleotides; no 9-deazaadenosine nucleotides were detected. (Supported by grants AI 27907 and AI 15663 from NIH, T16/181/T8 from WHO, and by the Burroughs Wellcome Company.)

C149 THE CERCARIAL PROTEASE OF SCHISTOSOMA MANSONI, James H. McKerrow, George Newport, Matthew Petitt, and Robert L. Lindquist, Naval Biosciences Laboratory, Univ. California Berkeley, Dept. of Chemistry, S.F. State Univesity, Department of Pathology, U. C., San Francisco, San Francisco, CA 94143.

Cercarial protease is a serine protease of 30,000kD molecular weight and pI 7.8 which does not bind to concanavalin A. It is secreted by cercariae in response to skin lipid, and degrades keratin, laminin, fibronectin, types IV and VIII collagens, elastin, and proteoglycan to facilitate invasion of skin by cercariae. The active site has been mapped and tetrapeptides with large hydrophobic or aromatic residues at P-1 are the best substrates. Ala-Ala-Pro-Phe-S-Bzl gives an approximate k_{cat}/K_m = 121,000 M^{-1} s^{-1}. Peptide inhibitor studies also suggest a hydrophobic pocket at P-4. Mechanism-based "suicide" inhibitors of other serine elastases are effective against the cercarial protease. A monoclonal antibody to the enzyme localizes it in the preacetabular glands of the cercaria and also mediates complement-dependent toxicity toward cercariae. A 20 amino acid N-terminal sequence shows no homology with other serine proteases. Northern blot analysis with an oligonucleotide probe based on the N-terminus shows protease mRNA synthesis only in the sporocyst stage (when cercarial glands are developing). When the protease is used as an antigen in ELISA, distinct high and low response groups are seen in sera from patients with schistosomiasis.

C150 LEISHMANIA DONOVANI AMASTIGOTES: METABOLIC PATHWAYS AND SUBSTRATE UTILIZATION, John C. Meade and Antony J. Mukkada, University of Cincinnati, Cincinnati, OH 45221

Amastigotes of Leishmania donovani possess all the enzymes of the Embden-Meyerhof pathway, phosphogluconate shunt and tricarboxylic acid cycle. Amastigotes utilize these pathways to catabolize glucose, palmitate, acetate and glutamate for the generation of energy and biosynthetic precursors. Label from [^{14}C] substrate appears in glycolytic and TCA cycle intermediates, amino acids and lipids, as well as in trichloroacetic acid insoluble compounds. The operation of the phosphogluconate shunt was demonstrated by following the evolution of [$^{14}CO_2$] from differentially labeled [^{14}C] D-glucose (C_1 and C_6 positions). Stimulation of endogenous amastigote respiration by these substrates is additional evidence for their utilization. Amastigotes were found to consume O_2 optimally at pH5.5 whereas promastigotes respire most efficiently at pH7.0. The ability of amastigotes to respire optimally under acidic conditions may represent an adaptation that reflects their acidic intracellular residence in phagolysosomal vacuoles. This adaptation appears to be at the membrane level since several enzymes in cell free separations show pH optima at or near neutrality.

C151 CHARACTERIZATION OF RIBONUCLEOPROTEIN PARTICLES OF TRYPANOSOMA BRUCEI, Shulamit Michaeli, Judith R. Levin, Kenneth P. Watkins, William J. Murphy and Nina Agabian. Naval Biosciences Laboratory, University of California, Berkeley 94720. Most, if not all T. brucei mRNA share a common 35 nucleotide 5' leader sequence. The source of this spliced leader (SL) appears to be a 135 base RNA species (SL-RNA) transcribed from a 1.4Kb repeating unit which is in most cases physically unlinked to the mRNA coding gene. We are characterizing the organization of the SL-RNA mRNA's and their respective precursors (hnRNA) with its nucleoprotein (RNP) particle in order to gain insight in the mechanism by which mRNA's acquire the SL sequence. Sucrose gradient analysis of nuclear RNP's revealed two peaks containing RNA hybridizing to an SL probe. One peak sedimenting at about 30S contains RNA that hybridizes with both VSG and tubulin probes as well as the SL probe and appear to correspond to the hnRNP particles found in other eukaryotic systems. The T. brucei particles contain proteins in the same molecular weight range as the hnRNP "core" proteins of vertebrates. In addition, both T. brucei and L. collosoma nuclei contain proteins that cross-react with monoclonal antibodies directed against 68 Kd hnRNP protein from HeLa cells (provided by Dr. G. Dreyfuss). The second peak hybridizing to the SL probe, sedimenting at about 10S', and appears to contain the SL-RNA; a complex with similar sedimentation properties is reconstituted when SL-RNA is incubated with nuclear proteins in vitro. We are currently characterizing the proteins associated with the SLRNP particle.

C152 ANTIGIARDIAL ACTIVITY OF ARABINOSYLADENINE AND ARABINOSYLGUANINE, R.L. Miller*, D.J. Nelson*, T.A. Krenitsky*, R.L. Berens# and J.J. Marr#, *Burroughs Wellcome Co., Research Triangle Park, NC 27709 and #University of Colorado Health Sciences Center, Denver, CO 80262

Giardia lamblia is the most common protozoal parasite infecting the small intestine in man. Previous studies by Wang and Aldritt [1] indicate that Giardia lamblia, which is incapable of de novo purine biosynthesis, incorporates purine nucleosides via hydrolytic cleavage followed by phosphoribosylation of the resultant purine. Although this appears to be the predominant route of nucleoside salvage in this organism, a recent report by Berens, et al. [2] indicates that 9-deazaadenosine, a non-hydrolyzable purine nucleoside, is phosphorylated. This observation implies that at least some purine nucleoside analogs may be directly phosphorylated. We would like to report the in vitro antigiardial activity of arabinosyladenine (AraA) and arabinosylguanine (AraG) and the phosphorylation of these compounds to their respective 5'-nucleotides. Growth studies using logarithmically growing cells in culture indicated that AraA (± dCoF, an adenosine deaminase inhibitor) and AraG had equivalent ED_{50} values of 4 μM. Whole cell incubations indicated that [^{3}H]AraA (+ dCoF) and [^{3}H]AraG are converted to their respective 5'-mono-, di-, and triphosphates. Essentially no [^{3}H]ribonucleotides were derived from either of these compounds. In addition, no AraG containing nucleotides were derived from AraA. Similarly, no AraA containing nucleotides were formed from AraG. These observations imply that AraA and AraG are not cleaved to their bases prior to phosphorylation and that there is essentially no interconversion of the AraA and AraG nucleotides. When tested at their 2x ED_{50} concentrations, neither AraA nor AraG had any effect on the ATP or GTP pool sizes of the intact organism in 24 hrs.

1. C.C. Wang and S. Aldritt (1983) J. Exp. Med. 58, 1703-1712.
2. R.L. Berens, D.L. Looker and J.J. Marr (1985) Fed. Proc. 44, 1333.

C153 CHARACTERIZATION OF A STAGE SPECIFIC GENE FROM TRYPANOSOMA BRUCEI. Jeremy C. Mottram and Nina Agabian, Naval Biosciences Laboratory, University of California, Berkeley, 94720. Malate dehydrogenase is developmentally regulated in T. brucei. On transformation from the bloodstream to the procyclic stage of the parasite there is an 8-fold increase in activity that is attributed mainly to the induction of a glycosomal isoenzyme. We have used antibodies raised against purified Leishmania mexicana amastigote malate dehydrogenase to screen a T. brucei gambiense lambda gt11 expression library. A positive expression clone was isolated that yielded a 1.3 Kb cDNA insert which hybridizes to 2 restriction fragments on T. brucei genomic DNA. Furthermore, one portion of the cDNA probe hybridizes to both of these copies whereas another portion hybridizes to only one. These data and additional restriction endonuclease mapping of the insert and genomic DNA indicates the presence of two genes. Genomic equivalents of these two gene sequences have been isolated and will be used in further investigating the structure and organization of the putative malate dehydrogenase genes and their transcripts.

C154 TRANSCRIPTIONAL ANALYSIS OF THE TUBULIN GENES OF TRYPANOSOMA BRUCEI, William J. Murphy, Shulamit Michaeli and Nina Agabian, Naval Biosciences Laboratory, University of California, Berkeley, CA 94720. RNA blot analysis of the tubulin genes of T. brucei has revealed an RNA species of larger molecular weight than that of the mature α and β mRNAs. This putative "precursor" RNA is polyadenylated and hybridizes with both the α and β tubulin gene probes. The size of this RNA suggests that it may arise either from transcription of an entire tubulin repeat unit, which is 3.7 kb and contains both the α and β genes, or from the processing of a much larger transcript (eg. the entire tubulin gene cluster). Either of these possibilities would indicate a polycistronic precursor RNA which is subsequently processed to yield the mature mRNAs. We are currently examining this putative precursor molecule for i) the organization of the α and β genes within, ii) the presence or absence of the tubulin intergenic sequences and iii) the presence or absence of the 35 base spliced leader and/or the 135 base spliced leader small RNA sequences.

C155 ROLE OF THE HOST CELL CLANTHRIN IN THE INTERIORISATION OF TRIPANOSOMA CRUZI A.Osuna.,S.Castanys and C.Alonso.Dept.Parasitol.Univ.Granada.Spain.
Tripanosoma cruzi has an intracelular multiplication phase adopting in the host vertebrate the form of an amastigote.During the interiorisation period both the parasites and the host cells play an active role.The importance of the cytoskeleton of the host cell has been studied by several authors using such compounds as cytochalasin B,latrunculin B and poly-L-lysine.All these studies suggested that the parasite interiorisation is a complex process similar to a macropycnocitic phenomenon.
We have studied the implication of the membrane associated protein,clanthrin,during the internalisation of the parasite.We have observed that the adherence and internalisation of the metacyclic forms to cultured Hela cells in vitro,treated with anti-clanthrin monoclonal antibodies,(1:1000),is significantly reduced relative to that of the control non-treated host cells,(Adherence index of 6.38 ± 1.2 relative to 13.8 ± 2.1 ;parasitation index of 0.84 ± 0.21 relative to 6.10 ± 1.13).This result suggests that clanthrin and other membrane associated proteins may also play an active role in the parasite host cell invasion.

C156 A RIBOSE TRANSPORT SYSTEM IN LEISHMANIA DONOVANI PROMASTIGOTES, Katie B. Pastakia and Dennis M. Dwyer, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD.
A transport system for ribose in L. donovani promastigotes was characterized by measuring the uptake of radioactively labelled sugar. 2-deoxyribose, D-xylose and L-arabinose inhibited ribose uptake whereas hexoses, adenosine and proline did not inhibit uptake, indicating that the transporter exhibited substrate specificity for certain pentose sugars. Intracellular ribose exchanged with 2-deoxyribose. Uptake of ribose showed saturation kinetics with an apparent K_m = 2mM and V_{max} = 6nmol/mg protein/min. N-ethylmaleiimide and p-hydroxymercuribenzoate inhibited ribose uptake which was reversed by dithiothreitol. Uncoupling agents and other inhibitors of energy-driven transport systems had no effect on ribose uptake. Following uptake, the intracellular pool had two thirds of the sugar in the phosphorylated form and one third in the unaltered form. These cumulative results suggest that a specific carrier mediates ribose uptake in L. donovani promastigotes and the mechanism of uptake is by facilitated diffusion.

C157 PURIFICATION AND CHARACTERIZATION OF A MAJOR SURFACE ANTIGEN OF EIMERIA TENELLA. Leland S. Paul[1], Virginia M. Brothers[2], James G. Files[1], John L. Tedesco[2], Karel Z. Newman[2] and Thomas C. Gore[2]. [1] Codon, 430 Valley Drive, Brisbane, CA 94005, and [2] Salsbury Laboratories, Inc., Charles City, IA 50616.

E. tenella is a species of Coccidia that leads to severe economic losses to the poultry industry. We have identified a major surface protein antigen of E. tenella sporozoites and sporocysts by immunoblotting and immunoprecipitation experiments. We have extracted this antigen protein from sporocyst membranes, purified it chromatographically, and determined the amino acid sequence of the component polypeptide chains. We have immunized chickens with the purified protein and have demonstrated that it is capable of raising antibodies that neutralize E. tenella sporozoites in vitro.

C158 CHARACTERIZATION OF THE 5' CAPS OF T. brucei AND L. collosoma mRNAs AND SL RNAS, Karen Perry and Nina Agabian, University of California, Berkeley, CA 94720.

All mRNAs ot T. brucei that have been studied have a common 5' terminal sequence of 35 nucleotides. This leader sequence appears to be acquired from the 5' end of a separately transcribed RNA of approximately 135 nucleotides which is called the spliced leader RNA or SL RNA. Previous studies which indicate that the SL RNA contains a 5' cap structure have suggested that this cap is donated to mRNA during its synthesis and/or processing. In order to characterize the T. brucei mRNA and SL RNA caps, we have developed methods for the purification of procyclic form polyA$^+$ and SL RNA labeled uniformly in vivo. These RNAs were digested in parallel with RNase T2 and the digestion products separated by PEI cellulose thin layer chromatography. The polyA$^+$ and SL RNAs appear to contain a common RNase T2 resistant structure with a mobility similar to that of known cap structures. The nature of these RNase T2 resistant structures is being investigated further. The polyA$^+$ and SL RNAs of the insect parasite, L. collosoma, also contain RNase T2 resistant structures with mobilities similar to those of known caps on PEI cellulose TLC. The SL RNA appears to contain a single RNase T2 resistant structure with a mobility which is identical to that of a polyA$^+$ RNA structure. PolyA$^+$ RNA contains additional cap-like structures not found on the SL RNA. We are currently examining the possibility that these additional structures are found on mRNAs that have either undergone further modifications or do not contain the leader sequence.

C159 SURFACE LABELING OF VSG ON VIABLE AFRICAN TRYPANOSOMES Jeffrey A. Radding, Grant T. Shimamoto, Thomas M. Lalor, William H. Konigsberg and Frank F. Richards, Yale University School of Medicine, New Haven, CT 06510

The surface accessible structural elements of five variant surface glycoproteins (VSGs) of African trypanosomes are probed by radioiodination of tyrosyl residues on live trypanosomes. In addition, two of the five VSGs are radiolabeled using ethyl and isethienyl acetimidates. These reagents are specific for primary amino groups. The labeled VSG is then isolated, reduced and cleaved to prepare a peptide mixture which is separated by RP-HPLC. Labeled peptides are then sequenced by automated Edman degradation and analysis. The labeled peptides are placed within the known sequence of the VSG. The surface accessible regions determined by this method are then correlated with the secondary structural potential of the VSG. Information generated by this technique indicate that surface accessible elements in different VSG molecules map to different regions in these proteins and these regions generally exhibit weak or variable structural potentials. These results support the concept of multiple folding patterns in the polygene family of VSGs and may be the mechanism by which successive VSG molecules present unique determinants on the surface of the trypanosome.

C160 BIOCHEMICAL MARKERS OF THE CELL DIFFERENTIATION OF TRYPANOSOMA CRUZI. R. Rangel-Aldao, G.Comach, O.Allende and F. Triana, BIOMED, U. de Carabobo, La Morita,Maracay, and R.Piras, M.Piras, & .D.Henriquez, Centro Medico La Trinidad, Caracas,Venezuela. To define specific and identifiable biochemical markers for epimastigotes (epis) and trypomastigotes (tryps) of T. cruzi we have: 1) established their respective polypeptide and antigenic maps by electrophoresis in polyacrylamide gels (SDS-PAGE), and immunoblotting with either monoclonal antibodies against cytoskeletal proteins or sera from chagasic individuals and; 2) studied intracellular signals involved in the differentiation of lower eukaryotes e.g. cAMP. Approach #1 revealed extensive homology in the SDS-PAGE maps of epis and tryps, the differences being mostly quantitative as determined by laser densitometry. Tryps showed a stage-specific band of 75K which was antigenic with chagasic sera. Immunoblots showed that both alpha- and beta-tubulin were more abundant in tryps than in epis. Immunoblotting with chagasic sera also revealed homology for both forms. Upon antigenic dilution, a cluster of bands of Mr=150K to 75K prevailed in the tryps, whereas epis displayed more abundance of antigenic bands of Mr=72K to 36K. Approach #2 showed an association of cAMP levels with the stage of differentiation of T. cruzi. Tryps contained 4x more cAMP than epis and a 2.5x higher activity of a unique type of cAMP-binding protein (CARPT), which behaved physically and kinetically identical in both forms of T. cruzi. CARPT apparently changes its subcellular location upon cell differentiation since it was mostly cytosolic in the epis and became associated with the nuclear fraction in the tryps.

C161 Isolation and Characterization of Glycosaminoglycans from Schistosoma mansoni. Nancy P. Robertson*and George D. Cain. Department of Biology University of Iowa, Iowa City, IA,52242

Tegumental tissues of paired adult Schistosoma mansoni were removed by treatment with Triton X-100 and recovered by centrifugation. The chloroform-methanol insoluble residues of this isolated tegumental fraction and of the denuded carcasses were analysed for glycosaminoglycan(GAG) and sialic acid contents.

Treatment with GAG-specific enzymes followed by electrophoretic analysis showed that both the carcass and tegument contained heparin and/or heparan sulfate, chondroitin sulfate and hyaluronic acid. All except hyaluronic acid were present in the tegumental fraction.

Based on uronic acid content, about 73% of the total GAG was in the tegumental membrane, 15% in the tegumental matrix and the remaining 12% were in the carcass.

The presence of heparin-like polysaccharides amy prevent entrappment of the schistosome by the host's blood-clotting process.

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EXPRESSION OF THE 'KNOB-ASSOCIATED' HISTIDINE-RICH PROTEIN OF PLASMODIUM FALCIPARUM CAN BE DISSOCIATED FROM THE CYTOADHERENCE PHENOMENON, Edwin Rock, Diane W. Taylor and Russell J. Howard, LPD, NIAID, NIH, Bethesda, MD 20892 and Georgetown University, Washington, D.C. 20057.

Knob-positive (K+) P. falciparum parasites adapted to continuous in vitro growth in human erythrocytes may retain the cytoadherence property (i.e. binding of infected erythrocytes to endothelial cells) or lose their binding capacity. We describe these parasites as K+B+ and K+B- respectively. An unusual histidine-rich protein (HRP: Mr 80,000-100,000) has been associated phenotypically with expression of knobs since all K+ parasites so far examined synthesize this protein. We compared K+B+ and K+B- parasites for expression of the 'knob-associated' HRP to test whether its expression is linked directly to expression of cytoadherence at the infected erythrocyte membrane. Three antibody reagents were used in immunofluorescence (IFA), immunoprecipitation and Western blotting experiments: 1. a mouse monoclonal antibody which reacts by IFA with all K+ but no K- parasites examined to date and which specifically reacts with the Mr 80,000-100,000 HRP from K+ parasites; 2. a rabbit antiserum against the purified 'knob-associated' HRP of K+ Malayan Camp strain P. falciparum; 3. a rabbit antiserum against the P. lophurae HRP. Two K+B+ parasites (clone $1tG_2F_6$ and V1) and four K+B- parasites (K3, L, N1 and T2) were compared. All parasites synthesized a 'knob-associated' HRP in the Mr range 80,000-100,000. Thus, expression of knobs and the 'knob-associated' HRP by K+B- parasites is not sufficient to confer cytoadherence.

C163 PROTEIN CHANGES IN THE LIFE CYCLE OF TRYPANOSOMA BRUCEI, Stuart Z. Shapiro and Bruce E. Kimmel, ILRAD, Nairobi, Kenya.

The protein products of differential gene expression form the physical basis for the morphological and biochemical characteristics of different stages in the parasite life cycle. We have used two-dimensional polyacrylamide gel electrophoresis to analyse changes in protein content and protein synthesis in three stages of the T. brucei life cycle: slender and stumpy bloodstream forms and the procyclic, vector midgut form. 2D gels were stained with ammoniacal silver to analyse protein content while 2D gels of (^{35}S) methionine labelled proteins were examined to analyse newly synthesized protein. Also, the in vitro translation products of mRNA purified from the three stages were analysed. Several stage specific proteins were noted. The most obvious is the VSG which is only present in bloodstream forms. Some other proteins were also bloodstream form specific. Some proteins were present both in stumpy forms and procyclics but not in slender forms. Several proteins were present only in one of the three stages. One protein present in both slender and stumpy parasites did not appear to be synthesized in the stumpy stage. Also, there appeared to be more mRNA in slender parasites for a protein which was greatly increased in synthesis and concentration in stumpy parasites; the synthesis of this protein may be under translational control.

C164 MOLECULAR MODIFICATIONS OF THE ERYTHROCYTE MEMBRANE BY MALARIA, I. W. Sherman and J. Greenan, Department of Biology, University of California, Riverside, CA 92521

Infection of an erythrocyte with the human malaria Plasmodium falciparum results in a developmentally regulated alteration in the membrane of the host cell: formation of surface excrescences, called knobs. Knobs are antigenically distinct from the remainder of the red cell, and are believed to be responsible for the adherence of malaria-infected cells to the venous endothelium. Changes in the distribution of red cell surface saccharides and anionic charge during intraerythrocytic development of P. falciparum were visualized using transmission electron microscopy and surface labeling. Aggregated ferritin particles, restricted to the protrusion of the erythrocyte membrane, were observed with human erythrocytes after exposure to cationized ferritin (CF) and ferritin-concanavalin A (conA). The remainder of the red cell surface in knobby erythrocytes, or the entire surface of uninfected red cells and cells infected with a knobless variant demonstrated a uniform distribution of CF, and an absence of aggregated conA particles. A dense uniform surface distribution of ferritin was observed with ferritin-wheat germ agglutinin and ferritin-ricin, and no preferential aggregation of ferritin particles in the knob region was in evidence with these lectins. Using Cancer antennarius and Limax flavus lectins no knob-associated change in the distribution of sialic acid residues, or modification of sialic acid by o-acylation, was found. These observations suggest that redistribution of surface charge as well as saccharide clustering on the erythrocyte surface are both parasite-induced and knob-related. (Supported by NIH grant AI 20456.)

C165 SEQUENCE HOMOLOGY AMONGST A NUMBER OF MEMBRANE PROTEINS OF BABESIA RODHAINI
David Snary, Wellcome Research Laboratories, Beckenham, Kent, U.K.

Babesia rodhaini is a tick-borne parasite which invades and multiplies within rodent red blood cells. Four membrane proteins of the parasite have been identified by monoclonal antibodies. These proteins are differentiated by peptide mapping, serological criteria and by molecular weight, but contain a region or regions of common amino acid sequence. A monoclonal antibody which reacts with a shared or common sequence also reacts to a mouse red blood cell protein suggesting that this common region may have an important role in the host-parasite relationship. The presence of this common sequence in several genes is also further evidence of gene duplication and/or gene rearrangement in Babesia species.

C166 **Experimental Leishmaniasis: Cyclosporin A prevents the development of cutaneous lesions in Balb/C mice.** W.Solbach, K. Forberg and M.Röllinghoff, Inst. Med. Microbiol., University Erlangen,Wasserturmstr.3, D-8520 Erlangen,F.R.G.

The influence of the T cell immunosuppressant Cyclosporin A (CsA) on the development of the local footpad swelling after murine infections with Leishmania tropica(L.tropica) was investigated. The aim of the study was to evaluate the contribution of T-lymphocytes to the initiation of the disease.
Subcutaneous injection of $2^{X}10^{7}$ living L. tropica promastigotes into the hind footpad of Balb/c mice causes local lesions with granulomatous inflammation within two weeks. Daily i.p. treatment with CsA(50 mg/kg) completely prevented the development of lesions for at least 7 weeks. 10mg/kg CsA still delayed the development of the lesions for about 10-12 days. Withdrawal of CsA resulted in prompt inflammatory responses in both groups indistinguishable in kinetics and magnitude from non-treated animals. Starting CsA-treatment 4 days after infection of the mice with L. tropica had no effect on the lesion. Since it is generally accepted that CsA mainly inhibits production/secretion of lymphokines by T helper cells, it is suggested that these cells or their soluble products are instrumental for the initiation of the L.tropica induced footpad swelling.

C167 PLASMODIUM CHABAUDI SUPPRESSION OF PRIMARY ANTIBODY RESPONSES TO SHEEP ERYTHROCYTES IN SUSCEPTIBLE AND RESISTANT MOUSE STRAINS, Mary M. Stevenson and Emil Skamene, The Montreal General Hospital Research Institute, Montreal, Quebec H3G 1A4

Immunosuppression is a well established characteristic of both human malaria and animal models of infection. Of major importance among human populations is the possibility of adverse effects on vaccination against malaria and other infectious agents. In considering the effect of malaria-induced immunosuppression on the outcome of vaccination, the influence of genetically determined host resistance or susceptibility to the initial episode of malaria may be of importance. We have, therefore, studied the development of suppression of the in vivo primary antibody response to sheep erythrocytes (S-RBC) during the course of infection with Plasmodium chabaudi in genetically resistant C57BL/6 and susceptible A/J mice. Spleen cells from both strains of mice immunized with S-RBC and infected on the same day showed significant increases in the number of plaque forming cells. The response of malaria-infected C57BL/6 mice was, however, significantly enhanced in comparison to both normal C57BL/6 and malaria-infected A/J mice. When mice were immunized at later times in the infection, the level of the response declined in both strains until the level was less than 50% of the normal response. The anti S-RBC response of susceptible A/J mice was suppressed until death at day 10. Similarly, the response of resistant C57BL/6 mice remained depressed through 40 days in spite of control and elimination of the parasite by day 18. Thus, the gene(s) controlling the level of resistance in mice to infection with P. chabaudi does not appear to influence whether or not suppression of the primary anti-S-RBC response develops.

C168 SELECTION OF A RICIN AGGLUTININ-RESISTANT CLONE OF LEISHMANIA DONOVANI DEFICIENT IN PHOSPHOGLYCAN, S. J. Turco and Diane L. King, Department of Biochemistry, University of Kentucky College of Medicine, Lexington, KY 40536

A variant cell line of Leishmania donovani has been selected for resistance to the cytotoxic lectin ricin agglutinin and shown to be defective in the synthesis of its major glycoconjugate (i.e. phosphoglycan). Compared to the parental line, the variant line continuously passaged in culture medium containing 0 µg/ml, 80 µg/ml, and 200 µg/ml of ricin exhibited, respectively, 45-, 90-, and 1,180-fold resistance to the toxic effects of the lectin. The synthesis of phosphoglycan (a 15-30 Kd polymer containing repeating units of phosphorylgalactosyl(β1,4)mannose as its salient feature) in the ricin-resistant clone was judged to be <1% relative to wildtype as determined by incorporation of radioactive mannose, galactose, and palmitate and by analyses on SDS polyacrylamide gels, Sephadex G-100, ricin agglutinin-bound agarose, and DEAE cellulose. Additionally, the variant line was observed to be several fold more sensitive to the lectin concanavalin A. The increase in sensitivity of the ricin-resistant clone to concanavalin A may be the result of an unmasking of glycoproteins due to the absence of phosphoglycan on the cell surface. These results suggest that variant line cannot assemble and express phosphoglycan and indicate that this variant cell line may prove useful in the study of the structure, biosynthesis, and function of this glycoconjugate. (This investigation received financial support from UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases and from a NIH RCDA AM01087 to S.J.T.)

Late Additions

C169 A FAMILY OF MEMBRANE CLYCOPROTEINS OF LEISHMANIA DETECTED BY MONOCLONAL ANTIBODIES, D.McMahon-Pratt and C.L.Jaffe, Yale University School of Medicine,New Haven,CT 06510, and Weizmann Institute of Science, Rehovot, Israel.

A number of monoclonal antibodies (T-1,T-8,M-2,M-4,M-6 and M-12) specific for distinct individual species of Leishmania by radioimmune binding assays recognize membrane components with a similar M_r (~50,000 daltons,nonreduced). Although the specificities of the monoclonal antibodies suggest that each molecule might be distinct,the similar molecular properties suggested that these molecules might in fact be related. Each of these glycoproteins appears to be a major surface radioiodinatable membrane component, which constitutes less than 2% of the membrane protein; upon reduction each of these proteins increased in M_r (to ~60,000 daltons). In addition, although the epitopes recognized by antibodies M-6,M-12 and T-1 are apparently species-specific for L. mexicana and L. major respectively and are not exposed within intact membranes, upon detergent solubilization (for immunoprecipitation) these determinants become exposed. Peptide mapping experiments employing metabolic and surface radiolabelled proteins demonstrate that the molecules recognized by M-2 and M-4 are identical but that these molecules are distinct from the molecules recognized by antibodies T-1, T-8, M-6 and M-12. Sequential immunoprecipitation experiments confirmed the fact that the epitopes recognized by these two groups of monoclonal antibodies reside on distinct sets of molecules. Comparative peptide mapping of the molecules recognized by antibodies T-1, M-6 and M-12 isolated from L. donovani and L. mexicana suggest that molecular variation occurs between species; these apparent differences may be due to glycosylation.

C170 STUDIES OF THE BIFUNCTIONAL THYMIDYLATE SYNTHASE-DIHYDROFOLATE REDUCTASE IN LEISHMANIA, Daniel V. Santi, Department of Biochemistry & Biophysics and Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143

In protozoa, thymidylate synthase (TS) and dihydrofolate reductase (DHFR) exist as a bifunctional protein (1). Leishmania tropica promastigotes have been developed which are resistant to the DHFR inhibitor, methotrexate (MTX), and the TS inhibitor 10-propargyl-5, 8-dideazafolate (CB3717) (2-4). The resistant organisms have high levels of the bifunctional TS-DHFR, and show amplified DNA sequences. The organisms are cross resistant to both MTX and CB3717. The amplified region of DNA designated as the R region, is about 30 kb in size and possesses regions of rearranged DNA sequences, which appear to be junctional regions. The amplified DNA in resistant organisms increases in amount as a function of the concentration of drug used, and initially appears as extrachromosomal circles; with increasing time or concentration of the drug, the amplified units of DNA appeared as higher molecular weight forms. The amplified DNA contains the gene encoding for the bifunctional TS-DHFR. In addition to TS-DHFR mRNA, three other abundant transcripts are found in resistant cells which hybridize to the amplified R-region DNA sequences. Three of four mRNAs are transcribed from the same strand of DNA, and appear to be clustered.

The bifunctional TS-DHFR from L. tropica has been purified to homogeneity and characterized (5,6). It has two identical subunits of $M_r \simeq 55$ kDA. The protein is extremely susceptible to proteolysis, whcih results in selective destruction of TS activity, and the generation of fragments of 36 kDa and 20 kDa. Limited proteolysis does not separate the bifunctional protein into its two component (TS and DHFR) domains. Rather, it cleaves at a highly vulnerable site of the TS sequence. Data on proteolysis experiments, amino acid sequencing and nucleic acid sequencing have provided information on the structure of the bifunctional protein. DHFR exists on the N-terminal region of the protein, whereas TS is found towards the carboxy terminal region. The sequence of L. tropica TS is extremely homologous to the corresponding enzymes found in other sources; in contrast, the sequence of DHFR is extremely variable. Other interesting aspects of TS-DHFR will be described.

1.Garrett, C.E., Coderre, J.A., Meek, T.D., Garvey, E.P., Claman, D.M., Beverley, S.M. & Santi, D.V., Molec. Biochem. Parasitol., 11, 257-265 (1984).
2.Coderre, J.A., Beverley, S.M., Schimke, R.T. & Santi, D.V., Proc. Natl. Acad. Sci. USA, 80, 2132-2136 (1983).
3.Beverley, S.M., Coderre, J.A., Santi, D.V. & Schimke, R.T., Cell, 38, 431-439 (1984).
4.Garvey, E.P., Coderre, J.A. & Santi, D.V., Molecular & Biochem. Parasitol., 17, 79-91(1985).
5.Meek, T.D., Garvey, E.P. & Santi, D.V., Biochemistry, 24, 678-686 (1985).
6.Garvey, E. P. & Santi, D.V., Proc. Natl. Acad. Sci. USA, 82, 7188-7192 (1985).

C171 State Specific Gene Expression during Trypanosoma cruzi Development.

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T. cruzi, the ethiological agent of Chagas' Disease, displays different morphological and functional types during its life-cycle. We have established a chemical defined in vitro differentiating condition for the study of the epimastigote to metacyclic trypomastigote transformation that occurs naturally within the insect host. This TAUP medium, consisting of triatomine artificial urine supplemented with 10mM L-proline has proven useful for the differentiation of several strains and clones of T. cruzi. The study of the gene expression products, during the transformation of the cloned Dm28c strain, has allowed the identification of stage specific polypeptides. Trypomastigote stage specific surface antigens of Mr 86,000 and 78,000 are primarily synthesized during the transformation process. A relationship has been established between synthesized proteins and trypomastigote stage specific biological properties. The mRNAs from both insect form differentiation stages have been characterized by their in vitro translation products and have been cloned, through their respective cDNAs, in the λgtll expression vector.

C172 PROTEOLYTIC ENZYMES IN EXTRACTS OF DIROFILARIA IMMITIS MICROFILARIAE.

A.L. Scott, W.K. Tamashiro and M. Rao. Johns Hopkins University, Baltimore, Md. 21205.

Experiments were conducted to investigate the role that proteases may play in the ability of filarial nematodes to avoid the immune response. Proteolytic activity in PBS extracts of D. Immitis microfilariae against Azocoll was highest at pH 7. Two SDS-resistant, mercaptoethanol-sensitive proteases were detected at 76 and 22 kD. The specificity of the proteases was determined by their ability to hydrolyze various substrates which were incorporated into the matrices of polyacrylamide gels. Using this technique, the 22 kd protease was shown to have activity against casine, fibrinogen, canine hemoglobin and canine IgG. Proteolytic activity of the 76 kD protease was limited to fibrinogen and IgG. Proteolytic activity was completely inhibited by PMSF indicating that both enzymes may be serine proteases. It was also demonstrated that live microfilariae have the ability to degrade canine IgG in vitro suggesting that proteoylitic cleavage of Ig may be one of the defense mechanisms that allow filarial nematode parasites to persist in the host.