MOLECULAR BIOLOGY OF HOST-PARASITE INTERACTIONS

Nina Agabian and Harvey Eisen, Organizers

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Parasite Genomes — Structure

0001 THE STRUCTURE AND REPLICATION MECHANISM OF KINETOPLAST DNA, Paul T. Englund, Stephen L. Hajduk, Peter A. Kitchin and Joan C. Marini, Department of Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, MD 21205

Kinetoplast DNA, the mitochondrial DNA of trypanosomatids, is a network of thousands of interlocked DNA circles. There are about 5000 minicircles in a network. The sequence heterogeneity and probable lack of transcription of minicircles suggests that they do not have a coding function. There also are about 20-50 maxicircles in a network. They contain genes similar to those in other mitochondrial DNAs.

Minicircle and maxicircle replication both occur during a discrete S phase of the cell cycle. Minicircle synthesis involves release of these molecules from the network, presumably by a topoisomerase, and then replication by a Cairns mechanism. The two progeny of each minicircle are then reattached to the network. Maxicircle synthesis involves rolling circle intermediates. These intermediates remain attached to the network, but after synthesis the tail is apparently released as a linearized maxicircle. Reattachment of these progeny molecules presumably involves threading through the network and recircularization. During the S phase the network grows in size, and when it reaches double size it splits in two. The two progeny networks, each identical to the parent, distribute to the two daughter cells.

An understanding of the function of kinetoplast DNA will require knowledge of how networks are organized within the mitochondrion. Electron micrographs of thin sections, obtained in many laboratories, indicate that networks have a highly organized compact structure in situ. We have recently found an unusual physical property of minicircle restriction fragments from Leishmania (and several other trypanosomatid species) that may be important to the intracellular organization of networks. Based on their behavior in gel electrophoresis, gel filtration, and electric dichroism experiments, these fragments appear to have an unusually compact structure presumably due to a natural curvature of the helix (1). This curvature is probably due to periodicities in the fragment's sequence. This natural bending of the molecule may facilitate assembly and stabilization of the network in its in situ conformation. The minicircles may therefore be structural elements, and the compact in situ form of the network may serve as a scaffolding for the cell's genetically important maxicircles. [Supported by NIH grant GM27608-14 and WHO grant 790184.]

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0002 GENOMIC ORGANIZATION OF MITOCHONDRIAL DNA IN LEISHMANIA, L. Simpson, A. M. Simpson, G. Kidane, M. Muhich, V. de la Cruz and N. Neckelmann, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

The mitochondrial DNA of Leishmania tarentolae and most other kinetoplastid protozoa is composed of thousands of minicircles and 20-50 maxicircles catenated together into a giant network of DNA. The maxicircle DNA is homogeneous whereas the minicircle DNA is heterogeneous in sequence. We have cloned and sequenced several L. tarentolae minicircles from different sequence classes in pBR322 and in M13 phage and have found a constant region of around 200 nt and a variable region. We also showed that the minicircle exhibits a weak ARS activity in a yeast plasmid, implying that the minicircles represent individual replicons. The maxicircle DNA in general can be isolated after release from the network by restriction enzyme digestion by virtue of its higher relative content of A + T. Intramolecular AT rich regions were visualized by partial denaturation mapping. Several maxicircle fragments were cloned in bacterial plasmids and M13 phage. Numerous poly A+ transcripts were mapped on the maxicircle in addition to the putative miniribosomal RNAs. Presumptive identification of four structural genes on the maxicircle was accomplished by low stringency hybridization of yeast petite DNA probes. Direct sequence analysis of these maxicircle fragments is in progress. A detailed comparison of the sequence homology of the 30 kb maxicircle DNA from L. tarentolae with the 22 kb maxicircle DNA from T. brucei has indicated that the relative organization of non-ribosomal genes is basically similar and that evolutionary changes have occured mainly by insertions/deletions within one region of the maxicircle. We conclude that the mitochondrial DNA of the kinetopastid protozoa has a unique genomic organization which nevertheless has some basic similarities with other mitochondrial genomes.

0003 CHARACTERIZATION OF MITOCHONDRIAL DNA IN AFRICAN TRYPANOSOMES. Kenneth Stuart. Issaquah Health Research Institute, 1595 N. W. Gilman Blvd., Issaquah, WA 98027

The large single mitochondrion of African trypanosomes contains two classes of circular DNA, maxicircles and minicircles, which share no sequence homology. About 50 maxicircles and over 5000 minicircles are catenated into a network.

All maxicircles, which are about 21 kbp in size, in a network are identical. We have cloned the entire maxicircle except for a 4.6 kbp segment representing about 20% of the maxicircle. The uncloned segment varies in size among stocks and has few restriction endonuclease cleavage sites. The maxicircle is largely but not completely transcribed. The non transcribed segment is adjacent to the uncloned variable region, part of which may also not be transcribed. The mitochondrial rRNA sequences have been localized in the maxicircle in addition to other genes which have been localized using cloned mitochondrial genes from other organisms. The nucleotide sequence of over half of the maxicircle has now been determined.

Minicircles, which are about 1 kbp in size, are very heterogeneous in sequence. This is a feature that distinguishes the African trypanosomes from other kinetoplastids. Trypanosoma brucei total minicircle complexity is about 300 kbp while it is much lower for other kinetoplastid flagellates. The number of different minicircles in a network is greater than 300 since different minicircles share sequence homology. Minicircle transcription has not been detected and nucleotide sequence analysis implies that minicircle function which is unknown may not include the production of a translation product.

Bloodstream trypanosomes lack cytochromes and a Krebs cycle while procyclic trypomastigotes which occur in the midgut of the insect host and can be cultivated have a fully functional mitochondrial respiratory system. Bloodstream forms contain the same maxicircle transcripts as do the procyclic forms indicating that the development of the mitochondrial respiratory system is not mediated by differential maxicircle transcription. Dyskinetoplastic mutants induced using DNA intercalating agents are unable to grow as procyclic forms and have mitochondrial DNA alterations. While some of these mutants are devoid of mitochondrial DNA some mutants retain reduced amounts of maxicircle and minicircle sequences.

Parasite Genomes — Regulation and Expression

0004 APPLICATION OF RECOMBINANT DNA TECHNIQUES TO THE HUMAN MALARIA PARASITE Plasmodium falciparum, J. Scaife, M. Goman, J.E. Hyde, R. Hall, G. Langsley, D. Simmons and J.W. Zolg, Department of Molecular Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, Scotland

We are studying the molecular biology of the human malaria parasite, Plasmodium falciparum. Our broad objective is to contribute knowledge and biological material which can be exploited to study aspects of the biology of this organism of potential clinical importance. Our present research focusses on the genetic material and products of specific genes. The genomic DNA has been purified from cultured erythrocyte stages of the parasite. It has the lowest G+C content (18%) known; DNA content is about 0.01 pg/nucleus (about 4x the genome of Escherichia coli) and has a significant fraction of repetitive sequences. The DNA has been cloned into phage λ.

The λ-based genomic library of P.falciparum DNA has yielded clones containing fragments of some interest (1). These include a repetitive DNA sequence which may contain a conserved element and which promises to provide a convenient way to distinguish parasites isolated from different locations. We have ribosomal RNA genes cloned. These are being analysed structurally to allow us to discover whether rDNA organisation in Plasmodium is related to that in other protozoa such as Dictyostelium, Physarum and Tetrahymena. We also have actin-specific sequences cloned, which are being analysed as models for gene expression.

Messenger RNA has been purified and its translation products documented. It has been converted to cDNA and cloned into another λ vector.

The genomic and cDNA libraries form the basis of the group's studies on gene products of the parasite. Those which are particularly interesting at present include enzymes of intermediary metabolism which are, or could be, good targets for antimalarial drugs. In addition we have made a set of monoclonal antibodies (McAbs) directed against parasite antigens. The McAbs are being used to define the antigens which are candidates for a vaccine. At the same time they can be used to screen for the cloned genes encoding immunologically important antigens. It is hoped that these efforts will lead to a means of synthesising large quantities of the antigen in an organism suitable for mass culture.

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Parasite Biochemistry

0005 PYRAZOLOPYRIMIDINE METABOLISM IN LEISHMANIA AND TRYPANOSOMES: SIGNIFICANT DIFFERENCES BETWEEN HOST AND PARASITE, J. Joseph Marr, Division of Infectious Diseases, University of Colorado Health Sciences Center, Denver, CO 80262

The pathogenic hemoflagellates of the genera Leishmania and Trypanosoma are major causes of human disease in the tropical and subtropical areas of the world. In general, the agents used to treat diseases caused by these organisms are toxic and not suitable for ease of administration to the millions of people infected. Investigations over the past several years have shown that there are several major differences between man and these protozoans with respect to purine metabolism. These differences appear to be promising for the development of effective chemotherapeutic compounds.These organisms do not synthesize purines de novo, as does man. They are able to concentrate pyrazolopyrimidines within the cell and metabolize them as purines through the salvage pathways, ultimately incorporating them into nucleic acids. This does not occur in mammals. The pyrazolopyrimidine base allopurinol, which has served as a prototype compound, is activated by a phoribosyltransferase to the ribonucleotide; The ribonucleotide is aminated, in the case of allopurinol, to the 4-aminopyrzolopyrimidine ribonucleotide and subsequently phosphorylated to the triphosphate form and incorporated into RNA. The pyrazolopyrimidine ribonucleosides formycin B and allopurinol ribonucleoside are activated by these protozoans through a nucleoside phosphotransferase. The resulting ribonucleotide is aminated and incorporated into RNA as described above. These metabolic peculiarities occur not only in the forms of these parasites which are found in the insect vectors but also in the intracellular forms which are pathogenic in man. These differences in the enzymology and metabolism of purines which exist in Leishmania and Trypanosoma offer excellent opportunities for chemotherapeutic exploitation.

0006 THE BIFUNCTIONAL THYMIDYLATE SYNTHETASE-DIHYDROFOLATE REDUCTASE IN PROTOZOA, Daniel V. Santi,* Jeffrey A. Coderre,* Stephen M. Beverley† and Robert T. Schimke†, *Departments of Biochemistry and Pharmaceutical Chemistry, University of California, San Francisco, CA 94143, and †Department of Biological Sciences, Stanford University, Stanford, CA 94305.

Thymidylate synthetase from bacterial and mammalian sources is a dimer of subunit M_r ≈ 35,000; dihydrofolate reductase from these sources is a monomer with M_r ≈ 20,000. Recently, both of these enzyme activities have been shown to exist on the same protein in *Crithidia fasciculata* and *Plasmodium berghei* (1). The bifunctional protein from *C. fasciculata* is a dimer of subunit M_r = 56,700. We have now obtained evidence that the higher molecular weight thymidylate synthetase-dihydrofolate reductase bifunctional protein may be a common and unique feature of parasitic protozoa. We have determined the M_r's of the thymidylate synthetase-dihydrofolate reductase from a variety of protozoan sources. The proteins from *C. fasciculata, Leishmania tropica, L. mexicana, L. braziliensis* and *Trypanosoma cruzi* have subunit M_r's of 56,000; the bifunctional proteins from *Plasmodium falciparum* and *P. lophurae* have subunit M_r's = 70,000 and that from *Eimeria tenella* shows M_r = 100,000. The M_r's of the native proteins from the aforementioned are approximately two-fold higher than the subunit M_r's, and we presume that all exist as dimers.

We have developed strains of *L. tropica* promastigotes which are highly resistant to methotrexate (2). In a strain resistant to one millimolar methotrexate (2000 times the wild-type EC_{50}), the thymidylate synthetase-dihydrofolate reductase bifunctional protein is present at 40-fold higher levels than in the wild-type organism. The enzyme overproduction is accompanied by amplification of specific regions of DNA and current evidence indicates that drug resistance is a result of gene amplification. When methotrexate is withdrawn from drug-resistant *L. tropica*, levels of the bifunctional protein and amplified regions of DNA decrease to that observed in the wild-type cells within about one hundred generations. However, re-exposure of such cells to very high concentrations of methotrexate does not result in cell-death, and resistant organisms possessing both increased levels of the bifunctional protein and amplified regions of DNA emerge within four to five generations.

The implications of these findings to chemotherapy and drug resistance in parasitic protozoa will be discussed.

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0007 DNA TOPOISOMERASE(S) OF TRYPANOSOMES : INHIBITORY EFFECTS OF SOME CHEMICALS, Guy Riou, Sétha Douc-Rasy, Alain Kayser, Laboratoire de Pharmacologie Moléculaire, Institut Gustave Roussy, 94805 Villejuif Cedex, France.

Kinetoplast DNA (kDNA) is composed of catenated circles. In T. cruzi a few maxicircles are tangled among some 25000 minicircles. Minicircles must undergo replication which is not straightforward, due to both the condensed state of the kDNA and the intricacy of the network. Topoisomerases are suspected to enter in play (1).
We succeeded in preparing an extract of T. cruzi nuclei that presented topoisomerase activities (2) : an enzymatic activity converted supercoiled DNA molecules into relaxed ones as appeared on electrophoretic analysis . Another activity was responsible for the formation of catenanes reminiscent of native kDNA, from circular DNA molecules. Both activities could be related to the same enzyme, a question that is presently under study. We have chromatographed the nuclear extract on hydroxyapatite columns and eluted a relaxing activity around 0.5 M K phosphate. This activity was ATP independent, was activated by spermidine, and relaxed positively as well as negatively supercoiled DNA molecules. The molecular weight of the enzyme was estimated to about 62 K.Those results are similar to data obtained on type I topoisomerases of other eukaryotic cells.
We have initiated an in vitro investigation on the effects on the topoisomerisation reaction of known trypanocidal agents. Intercalating drugs (ethidium, acridine and ellipticine derivatives) were shown to inhibit the topoisomerisation. Dimeric derivatives of the same drugs were even more active. It is known that ethidium bromide and other drugs, when added at sublethal concentration to some cultures of trypanosomes, induced the appearance of dyskinetoplastic cells. In other words, replication of kDNA was preferentially inhibited in the treated cells. Our hypothesis is that topoisomerase(s) molecules could no more react in vivo with their kDNA substrate.

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Non-Immune Mechanisms of Parasite Killing

0008 EFFECT OF EXOGENOUS ELECTRON CARRIERS ON INTRACELLULAR PARASITE KILLING, Jacques W. Mauel, WHO Laboratories, Institute of biochemistry, University of Lausanne, CH-1066 Epalinges, Switzerland

Leishmania parasites are obligatory intracellular protozoa that live in cells of the mononuclear phagocyte system. Recovery from leishmanial infections is thought to involve macrophage activation (1,2). Experiments in vitro have shown that incubation of Leishmania-infected mouse peritoneal macrophages with lymphocyte mediators (such as macrophage activating factor, MAF) results in destruction of the intracellular microbes (3). In addition, exposure of parasitized cells to certain electron carriers similarly induces intracellular killing (4,5). Differences and similarities between both phenomena have been examined.

Activation by MAF leads to progressive intracellular parasite destruction reaching completion within 20 h; stimulation of the hexose phosphate shunt (HPS) follows similar kinetics, and parasite killing is thought to depend on the generation of toxic oxygen metabolites by the activated phagocytes (2,6).Considerable differences appear to exist between mouse strains in the capacity of their macrophages to respond to MAF by increased leishmanicidal activity. In comparison, exposure of Leishmania-infected macrophages to electron carriers induces intracellular parasite destruction within seconds. The concentrations of the drugs required to achieve this effect are far below those necessary to obtain killing of free parasites, and the kinetics of both processes are very different. Electron carriers also produce an immediate stimulation of the HPS and oxygen uptake. Moreover, macrophages from different genetic backgrounds appear to display a similar killing activity towards all Leishmania species tested. Killing of intracellular Leishmania in MAF- and electron carrier-stimulated macrophages are both inhibited by cytochrome C, a scavenger of superoxide. Cytochrome C also reduces HPS stimulation. The same compound has little effect upon the toxicity of electron carriers for extracellular parasites, however. These observations suggest that electron carriers induce intracellular parasite killing as result of stimulation of oxidative processes in the host-cells, and thus mimic in some respects the parasiticidal activity of MAF-treted macrophages.

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0009 PHAGOCYTES AND OXYGEN-DEPENDENT ANTIPARASITE SYSTEMS, Richard M. Locksley, Department of Medicine, University of Washington, Seattle, WA 98195

Perturbation of the membrane of phagocytes results in a burst of oxygen consumption and the generation of superoxide anion (O_2^-). Although itself relatively nontoxic, its dismutation product, hydrogen peroxide (H_2O_2), and the products of the iron-catalyzed interaction of O_2^- and H_2O_2, have powerful microbicidal capacities (1). Further, granule peroxidase, either myeloperoxidase (MPO) or eosinophil peroxidase (EPO), markedly augments the toxicity of H_2O_2 in the presence of a halide. Although a potential role for phagocyte oxygen-dependent microbicidal systems has been demonstrated against diverse parasites, the complex interplay between phagocytic cells and parasite defense mechanisms is well illustrated by the obligate intracellular protozoa - Toxoplasma, Leishmania, Trypanosoma - which parasitize the resident macrophages (Mϕ) of the mononuclear phagocyte system. In cell-free systems, the MPO (or EPO)-H_2O_2-halide system is highly toxic to these organisms. Likewise, neutrophils (PMN), which undergo a vigorous respiratory burst with phagocytosis and contain substantial amounts of peroxidase, are protozoacidal. When they have been investigated, PMN from patients with chronic granulomatous disease, and to a lesser extent, from patients with MPO-deficiency, kill these organisms less efficiently. Freshly isolated blood monocytes, which undergo a less vigorous respiratory burst upon phagocytosis of these protozoa and contain substantially less granule peroxidase than do PMN, still kill up to 90% of ingested T. gondii and Leishmania promastigotes and restrict the replication of surviving organisms. Human blood monocytes maintained in tissue culture lose granule peroxidase and the respiratory burst becomes markedly attenuated; such cells resemble the tissue Mϕ. This severe deficit in oxygen-dependent microbicidal activity correlates with an inability of these cells to kill or restrict the intracellular replication of protozoa (2). Enhancement of antiparasitic activity by Mϕ can be achieved either by activation of Mϕ by exposure to sensitized T-cell products (3), which results in augmentation of the respiratory burst, or by the introduction of exogenous peroxidase into the vacuole, a mechanism whereby the toxicity of the small amounts of H_2O_2 formed may be amplified (4).

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Mechanisms of Parasite Escape from Host Defense Systems I — Antigenic Variation

0010 THE DIVERSITY OF ANTIGENIC VARIATION, John J. Doyle, ILRAD, P.O. Box 30709, Nairobi, Kenya

The phenomenon of antigenic variation can be defined as the ability of an organism to express different genes or altered genes, the products of which allow survival in hostile environments. This phenomenon is best exemplified by the ability of salivarian trypanosomes to survive in the bloodstreams and tissue fluids of their mammalian hosts by continually changing their surface antigens exposed to the host immune response (1). Other parasitic protozoa, such as Plasmodium and Babesia spps, have an intra-erythrocytic location in the mammalian host, also vary parasite antigens displayed on the surface of infected erythrocytes to escape lytic host immune responses (1,2,3). Free living protozoa alter surface components in response to environmental changes (4). The phenomenon is not confined to protozoa. The haemagglutination antigen of influenza virus undergoes antigenic variation (5). Among the prokaryocytes, antigenic variation has been described in Salmonella, Neisseria, and Borrelia infections (6,7,8,9). Antigenic variants can have different physiological qualities which also allow survival in different environments (7,8,10). The antigens and genetic mechanisms underlying the phenomenon of antigenic variation have in some cases been described in detail (1,5,6,11) while in other cases little is known e.g. Borrelia. The process of antigenic variation in pathogenic organisms represents a considerable obstacle in some cases to the development of immunological control measures.

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0011 THE VARIANT ANTIGEN ON PLASMODIUM KNOWLESI-INFECTED ERYTHROCYTES,
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Erythrocytes infected with mature asexual stages (late trophozoites and schizonts) of the simian malaria parasite P. knowlesi express new antigens on their surface such that they are specifically agglutinated by sera from infected rhesus monkeys [1]. These antigens, called SICA-antigens (SICA = schizont-infected cell agglutination) undergo antigenic variation during chronic P. knowlesi infections [2] and it has been suggested that the parasite's capacity to vary the SICA-antigens allows it to evade variant-specific immune responses [2]. We have produced a clone of P. knowlesi [Pk1(A+)] and an antigenic variant of that clone that was recloned [Pk1(B+)1+], together with rhesus monkey and rabbit antisera that specifically agglutinate each clone [3]. The P. knowlesi variant antigens have been identified: 1. Infected cells were labeled by lactoperoxidase-catalyzed radioiodination, extracted in detergent and antigens immunoprecipitated with a panel of antisera of defined agglutination specificity. Antigens of M_r 190,000 & 210,000 with Pk1(A+), and M_r 200,000 (minor band) & 205,000 with Pk1(B+)1+ were only precipitated by sera which agglutinate each clone. 2. Intact radioiodinated cells were incubated with different sera, washed to remove unbound antibody, antigen-antibody complexes extracted with detergent and immunoprecipitated. The same ^{125}I-antigens were specifically precipitated from each clone. 3. Malarial proteins were biosynthetically radiolabeled by in vitro culture of parasites in the presence of ^{35}S-methionine. Intact schizont-infected cells were then incubated with various sera and cell surface antigens identified as for the second protocol. ^{35}S-labeled antigens of Pk1(A+) (M_r 190,000 & 210,000) and Pk1(B+)1+ (M_r 200,000) were again specifically precipitated only by agglutinating sera. We conclude that the intracellular malaria parasite exports an antigenically variable malarial protein to the surface membrane of the infected erythrocyte. Studies on the structural differences between these antigens and control of their gene expression are underway.

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0012 GENE REARRANGEMENTS CONTROLLING THE EXPRESSION OF SURFACE ANTIGEN GENES IN TRYPANOSOMES, P.Borst, A. Bernards, L.H.T. van der Ploeg, P.A.M. Michels, A.Y.C. Liu and T.De Lange, Section for Medical Enzymology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, P.O.Box 60.000, 1005 GA Amsterdam, The Netherlands.

The surface coat of African trypanosomes, like Trypanosoma brucei, consists of a single protein, the Variant Surface Glycoprotein (VSG). By switching from the synthesis of one VSG to the next, trypanosomes change the antigenic nature of their surface and escape immune destruction (see ref. 1). We have shown that one route for VSG gene activation involves the duplication and transposition of a silent Basic Copy (BC) gene into an expression site, yielding an expression-linked copy, that is transcribed [2-8]. The expression site is located at the end of a chromosome [4,5,9]. The 5' 35 nucleotides of the mature mRNA are not encoded in the transposed segment and must be derived from a mini-exon in the expression site [7]. The DNA sequence of one of the circa 100 mini-exons shows that pre-mRNA splicing follows the GT/AG rule.

A minor group of VSG BC genes is activated by a second route that does not involve gene duplication [1]. Like the VSG gene expression site, such genes are at the end of a chromosome [1,10,11]. We interpret our recent results to mean that these genes are transposed into the expression site by chromosome end exchange [10,12]. A single expression site could, therefore, control expression of the 1000-odd VSG genes [8] in a mutually exclusive fashion.

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Mechanisms of Parasite Escape from Host Defense Systems II — Sequestration

0013 THE SURFACE MEMBRANE CHEMISTRY OF LEISHMANIA: ITS POSSIBLE ROLE IN PARASITE SEQUESTRATION AND SURVIVAL IN HOST-CELL LYSOSOMES, Dennis M. Dwyer,Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD 20205 and Michael Gottlieb, Department of Immunology and Infectious Diseases, The Johns Hopkins University, Baltimore, MD 21205

Leishmania reside and multiply in hydrolytic environs throughout their developmental cycle (i.e. as extracellular promastigotes in the sandfly vector gut and as obligate intracellular amastigotes within phago-lysosomes of mammalian macrophages). These observations indicate roles for the parasite surface membrane not only in survival but also in macrophage recognition and subsequent targeting into the lysosome. Cumulative lectin binding and localization results have demonstrated the uniform distribution of mannose-ligands on intact L. donovani promastigotes and on the external face of their isolated surface membranes. The latter contain $\geq$20 mannose-glycoconjugates.Further, results of lectin-affinity binding studies using detergent extracts of surface radiolabeled intact cells demonstrated that the L. donovani surface membrane contained $\geq$ 17 externally oriented iodinable mannosylated-glycoconjugates. Recent evidence has indicated that some mammalian lysosomal enzymes are targeted, sequestered, and recycled to lysosomes via receptor-mediated processes involving their mannose-ligands. By analogy, some L. donovani surface membrane mannose-ligands could be recognized by similar macrophage mannosyl-enzyme receptors and thus mediate parasite uptake and sequestration into the lysosome. Moreover, such ligand-receptor mediated uptake might confer protection to the parasite from lysosomal digestion (i.e. recognition of the parasite surface as an "endogenous" lysosomal component). In this regard, the parasite externally oriented surface membrane bound acid phosphatase (a mannosyl-glycoprotein) might function in such processes. Similar speculations obtain regarding the soluble parasite-secreted acid phosphatase (also a mannosyl-glycoprotein). The functions of these two parasite enzymes with regard to infected lysosomal physiology remain to be elucidated. In addition to the latter enzymes, we have identified and characterized a distinct 5'- and a 3'-nucleotidase on the external surface of L. donovani pro- and amastigotes. As this organism requires preformed purines for growth, these two enzymes must play important functional roles in acquiring these bases from the host. Leishmaniasis might be considered as a living and multiplicative storage disease of macrophage lysosomes. In characterized lysosomal storage diseases, the metabolic lesions have been identified; whereas, those involved in leishmaniasis remain to be elucidated. However, our cumulative biochemical data imply that the surface membrane plays an important role in such parasite survival and "storage". Supported in part by USPHS Grant AI-16530 and a UNDP/World Bank/WHO Special Program TDR Grant both to M.G.

0013A INTERACTION WITH HOST CELLS IN TRYPANOSOMA CRUZI INFECTION, Leslie Hudson, Department of Immunology, St. George's Hospital Medical School, London SW17 ORE

After an initial parasitaemic phase T. cruzi infection in mice and man, soon declines to a level where parasites can only be detected with extreme difficulty using techniques that rely on parasite proliferation, e.g. haemoculture or xenodiagnosis. Parasites rarely infect neuronal cells and yet Chagas' disease is thought to arise by a neurogenic mechanism which may reduce cardiac neurones by 50%. Evidence has shown that the immune response to infection is involved in this cell destruction in 2 ways: a) parasite antigens may bind to host cells thus rendering them susceptible to the host's own antiparasite immune response (1) and b) parasite and host cells, in particular neurones and cardiac cells, share common antigens, as defined by monoclonal antibodies (2). It is suggested that the immune response shows a similar dichotomy, one component recognising parasite specific antigenic determinants, responsible for the resolution of acute stage parasitaemia, and a second component recognising cross-reactive antigens. Cross-reactive antigens on the parasite surface are sufficient to immunise the host, but cannot gain access to neuronal cell antigens until these cells have been permeabilised (2). It is suggested that adsorption of parasite antigens and a consequential attack by the host's own anti-parasite immune response may be sufficient to begin this "permeabilisation" process in vivo, thus exposing a leaky neurone to a pre-existing autoimmune response.

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2. Wood, J.N., Hudson, L., Jessell, T.M. and Yamamoto, M (1982). A monoclonal antibody defining antigenic determinants on subpopulations of mammalian neurones and Trypanosoma cruzi parasites. Nature 296 (5852): 34-38

Mechanisms of Pathogenesis

0014 MECHANISMS OF PATHOGENESIS IN E. COLI EXTRAINTESTINAL INFECTIONS, Stanley Falkow, Department of Medical Microbiology, Stanford University School of Medicine, Stanford, CA 94305

E. coli is the predominant facultative organism isolated from endogenous urinary tract infection (UTI) and septic conditions complicating abdominal wounds, peritonitis and appendicitis. It has become increasingly clear that only a relatively limited number of E. coli strains possessing "special properties" are most often associated with these extra-intestinal infections. In human UTI there is a high correlation between the ability of bacteria to adhere to uroepithelial cells and their virulence. The receptors on uro-epithelial cells to which the E. coli bind have been shown to be a disaccharide moiety of glycosphingolipids. We (1) have shown that a chromosomal locus, Pap, encodes for bacterial pili which mediate this binding. These pili are distinct from the "common" type I pili, Pil, which mediate binding to mannosides found on the majority of fecal E. coli. Homogenic pairs of strains differing only in the presence of cloned Pil or Pap gene sequences have been compared in an ascending mouse pyelonephritis model. Cells carrying the Pap sequences persist for a longer period of time and have a selective growth advantage in the kidney. Not only are uropathogenic strains of E. coli more likely to adhere to epithelial cells but a significantly greater proportion of these strains are found to be hemolytic (Hly), possess a ColV plasmid, exhibit resistance to the killing action of normal human serum and to belong to a limited number of O-antigenic serogroups. We (2) have examined the contribution of cloned Hly genes to the pathogenicity of E. coli in a rat intraperitoneal abscess model. The presence of the cloned chromosomal genes encoding Hly were sufficient to covert an avirulent fecal isolate to a strain capable of killing the majority of infected animals. Not all Hly genes cloned from animal or human E. coli strains confer pathogenicity; this difference has been found to be largely confined to a roughly 200 base pair AvaI fragment in the Hly operon.

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2. Welch, R.A. et al. 1981, Nature, 294:665.

0015 INTERACTIONS BETWEEN INTESTINAL CELLS AND *SHIGELLAE*, David Mirelman, Yael Nuchamowitz and Mordechai Izhar, Department of Biophysics and Unit for Molecular Biology of Parasitic Diseases, Weizmann Institute of Science, Rehovot 76100, Israel.

The adherence of pathogenic bacteria to host epithelial cells is regarded as a prerequisite for colonization and virulence manifestation. In most cases that were investigated the attachment of enteric bacteria to mammalian cells was mediated by bacterial cell surface appendages such as fimbriae, pili or flagella. Some of these appendages have been found to possess specific carbohydrate-binding (lectin) properties which enable them to recognize and attach to distinct receptor structures on the surface of epithelial cells. The adherence mechanism of non-pilated clinical isolates of *Shigella flexneri* to the intestinal mucosa of a number of animals was investigated. Guinea pig colonic mucosa, as well as isolated epithelial cells released by treating sections of the colon with solutions containing EDTA, dithiothreitol, and citrate avidly adhered *Shigella flexneri* bacteria. Separation of the intestinal cells from non-bound bacteria was achieved by differential sedimentation on a Percoll density gradient. Adherence of *S. flexneri* to the colonic cells was Ca^{2+} and time dependent, and almost no attachment (>5%) was observed at low temperature. The average number of bacteria which bound to colonic cells was 70 bacteria per cell, whereas attachment to cells isolated from the ileum region was 6 bacteria per cell. Colonic cells obtained from the intestines of rabbits, hamsters or rats did not adhere *Shigella*. Adherence to guinea pig colonic cells was inhibited (50%) by several carbohydrates, such as fucose (1 mg/ml) or glucose (5 mg/ml), as well as by the lipopolysaccharide (10 µg/ml) isolated from *S. flexneri* cells. Fixation of the bacteria with glutaraldehyde or preincubation of the bacteria with proteolytic enzymes did not affect their adherence. Proteolytic digestions or fixation of the epithelial cells, as well as pre-treatments with lipopolysaccharide or fucose solutions, abolished their ability to adhere bacteria. Moreover, extensive washings of the epithelial cells caused a marked decrease in their ability to bind bacteria and released a soluble substance which agglutinated a variety of *Shigellae* type cells as well as many strains of *E. coli*. The guinea pig intestinal agglutinin appears to be one of the components of the colonic mucus that coats the epithelial cells. Partial purification on a fucose-Sepharose affinity chromatography column indicates that it is a protein whose relative abundance is quite low (>1%) and the molecular weight of its subunits appear to be smaller than 60,000 daltons. These results clearly indicate that, in contrast to other bacterial adherence mechanisms, the adherence of *Shigella flexneri* is mediated by an intestinal carbohydrate-binding, lectin-like substance which binds to a carbohydrate that is most likely a component of the bacterial lipopolysaccharide. Drugs or metabolic conditions which are known to affect the outflow of mucus may affect the interaction of bacteria with colonic cells.

0016 GENETIC STUDIES ON SHIGELLA SPECIES ENTEROINVASIVENESS, Philippe J. Sansonetti, Service des Entérobactéries, U199 INSERM, Institut Pasteur, Paris, France.

Bacteria of the genus Shigella as well as some E. coli serotypes produce a dysenteric syndrome in humans. Their pathogenic potential correlates with their ability to invade the colonic mucosa and elicit a local inflammatory reaction which leads to abscesses and ulcerative lesions. No clear role has been ascribed so far to the Shiga toxin which is both enterotoxic and cytotoxic. Current experimental models comprise HeLa cell monolayers invasion, keratoconjunctivitis in the guinea pig, and rabbit ileal loop infection which is monitored for tissue invasion and fluid production.

A large plasmid of 120-140 Mdal has been demonstrated in all the virulent isolates belonging to Shigella and enteroinvasive E. coli serotypes (1,2,3). Inter-species transfer of this plasmid after transposon labeling confirmed its involvement in virulence. Hybridization experiments showed a large amount of homologous sequences between these virulence plasmids suggesting that they may derive from a common ancestor.

In a stepwise manner, E. coli K12 was conferred fully virulent properties upon transfer of chromosomal and plasmid genes from Shigella flexneri, thus demonstrating that virulence was determined by chromosomal and extrachromosomal genes functioning in concert. Plasmid genes appeared necessary for the very step of cell penetration whereas additional chromosomal genes were involved in tissue invasion, production of fluid and keratoconjunctivitis.

Studies on the molecular expression of this plasmid showed that it did not code for alterations in the bacterial lipopolysaccharide but that it did encode outer membrane proteins as demonstrated in minicells (4).

Based on these data, a general model for the genetic control of enteroinvasiveness by Shigella spp and enteroinvasive E. coli serotypes is presented.

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Parasite Recognition and Penetration of Target Cells

0017 MALARIA MEROZOITE INVASION OF ERYTHROCYTES, L. H. Miller, P. H. David, T. J. Hadley, D. E. Hudson, Malaria Section, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD 20205

Invasion of erythrocytes by malaria merozoites involves specific recognition between merozoite and erythrocyte, apical reorientation, junction formation, release of rhoptries with vacuolar formation and entry of the merozoite into the vacuole. Different erythrocyte determinants are involved in initial recognition and in junction formation, since P. knowlesi merozoites attach equally to refractory Duffy blood group negative human erythrocytes and Duffy positive erythrocytes. Junction formation does not occur with Duffy negative erythrocytes. We have now found that a monoclonal antibody to rhesus erythrocyte band 3, the major transmembrane glycoprotein, blocks invasion by P. knowlesi. Fab fragments of this monoclonal antibody also block invasion. The reorganization of intramembranous particles (band 3) in the junctional region may reflect binding of merozoites to this component. The evidence is building that P. falciparum binds to erythrocyte glycophorins (to be discussed by Dr. Jungery) and thus has a different receptor system than P. knowlesi. P. knowlesi and P. falciparum attach to major erythrocyte membrane proteins (band 3 and glycophoran, respectively) and invade erythrocytes of all ages. Is it possible that parasites that invade only young erythrocytes (e.g., P. vivax) utilize a receptor that decreases as the erythrocyte ages?

The merozoite surface components involved in invasion are unknown. Surface proteins on P. knowlesi merozoites have been identified by lactoperoxidase-catalyzed radioiodination and trypsin treatment and included proteins of m.w. 140,000, 105,000 and 75,000. The possibility that these were cleavage products of a higher m.w. protein was raised by a study of a monoclonal antibody to the surface of P. yoelii merozoites that precipitated proteins ranging in m.w. from 230,000 to 56,000 (Holder and Freeman, Nature 295:361, 1981). We have studied immunoprecipitates of a monoclonal antibody to a comparable molecule (250,000 m.w.) in P. knowlesi. We have observed that the protein is cleaved to discrete products by specific enzymes and will discuss the surface components of viable merozoites. To determine the relatedness of the 140,000 m.w. protein to other surface proteins, we immunized mice with liposomes containing merozoite proteins from the 140,000 m.w. region of the polyacrylamide gel. Whereas the anti 250,000 monoclonal antibody reacted around the entire merozoite

surface, the anti 140,000 reacted only at one end of the merozoite. It immunoprecipitated only the 140,000 m.w. protein from surface labeled merozoites and not the 250,000 m.w. protein or its cleavage products. The role of each of these surface molecules and possibly others as receptors for in vasion must now be defined so as to define the events during invasion at a molecular level.

0018 P. FALCIPARUM LECTIN-LIKE RECEPTOR BINDS TO RED CELL GLYCOPHORIN, Michele Jungery, Tropical Medicine Unit, Nuffield Department of Clinical Medicine, University of Oxford, Oxford OX3 9DU, UK.

Research to date has strongly suggested that the red cell membrane possesses receptors for malarial parasites and that these receptors are species specific (1). P. falciparum, the most important human malarial parasite can only invade red cells which contain the surface sialoglycoproteins (SGPs) glycophorin A, B and possibly C (2,3,4). Sialoglycoproteins both in solution and inserted into liposomes block parasite invasion of red cells. The parasites appear to bind to the red cells in a lectin-like fashion, since three monosaccharides, namely N-acetyl glucosamine - (Glu Nac), N-acetyl galactosamine - (Gal Nac), and N-acetyl neuraminic acid - (NANA) can specifically block parasite invasion in vitro. Moreover, Glu Nac, when coupled to bovine serum albumin, is particularly effective in blocking invasion at very low concentrations. Four to five proteins from a lysate of metabolically labelled P. falciparum schizonts bind to sepharose 4B - coupled SGPs containing the putative receptor for P. falciparum. Three of these proteins are sugar-specific in that they can bind to Glu Nac coupled sepharose 4B and can be eluted from both SGP and Glu Nac sepharose 4B columns with Glu Nac. The three sugar binding proteins appear to be structurally related to each other. The identity of the oligosaccharides and adjacent molecules bound by the proteins we have isolated may reveal which portion of the glycophorin molecule serves as the definitive merozoite attachment site.

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Cellular Interactions with The Host Immune System

0019 PARASITE KILLING AND IMMUNOSUPPRESSION BY MACROPHAGE PRODUCTS IN MALARIA,

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We have studied the role and mechanism of activation of macrophages in blood-stage murine malaria, using both lethal and non-lethal species of parasite in normal, T-depleted, and vaccinated mice. T-independent macrophage activation was found in all species of malaria tested, as measured by the secretion of plasminogen activator, by macrophage-mediated suppression of lymphocyte proliferation (1), and by the priming of macrophages to release Tumour Necrosis factor (TNF) on stimulation by endotoxin (2). However a role for T cells in the latter phenomenon was suggested by the fact that less endotoxin was required if T cell products were present.

Two separate macrophage secretory products, a TNF-like factor (2) and hydrogen peroxide (3), were shown to be cytotoxic to malaria parasites in vitro. Administration of these agents in vivo reduced parasitaemias in non-lethal infections, but had less or no effect on some lethal infections.

We suggest that macrophage products may contribute to the killing of blood-stage malaria parasites, and that lethality is partly associated with in vivo resistance to these products.

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(3) H.M. Dockrell, J.H.L. Playfair. "Killing of blood-stage malaria parasites by hydrogen peroxide". Infection & Immunity 1983 (in press)

0020 Characterization of Protective Antigens from Erythrocytic Stages of Plasmodium Falciparum. *Philippe Dubois, Serge Paulliac, Thierry Fandeur, Jean Pierre Dedet, and Luiz Pereira da Silva. Institut Pasteur, 75724 Paris Cedex 15 and Institut Pasteur de Guyane, 94300, Cayenne, France.*

Immunoglobulins were purified from serum or ascitic fluids of Saimiri monkeys infected with Plasmodium falciparum and with differing levels of functional immunity. Their protective activity was assessed in studies of passive transfer of immunity in vivo and their neutralizing activity was measured in studies of parasite inhibition in vitro, in culture of human or Saimiri red blood cells. Both protective and inhibitory antibodies were detected in different Ig preparations. The levels of these activities were however not directly correlated. Some Ig preparations, showing high protective activity, provide little or no inhibition of the parasite in vitro. Conversely, inhibitory activity was present in Ig preparations unable to confer protection in vivo. These results indicate that protective antibodies are active through a more complex immune mechanism than simple neutralization probably involving cellular immunity.

IgG preparations obtained from infected monkeys and presenting or not protective antibodies were used for the immunochemical analysis of parasite antigens. ^{35}S-methionine labelled parasite extracts from the schyzont stage of two different strains were used as antigen sources. Most of the parasite proteins were equally immunoprecipitated by the different IgG preparations analysed. However, some quantitative differences were demonstrated, particularly for two proteins of apparent MW approximately 76 Kb and 100 Kb respectively. IgG preparations with protective activity were shown to contain a considerably higher level of antibodies against both of these proteins. Serum of humans from hyperendemic areas and who are resistant to falciparum infection also present high levels of antibodies against the same proteins.

Parasite Biochemistry

0021 DNA AMPLIFICATIONS IN METHOTREXATE-RESISTANT LEISHMANIA, Stephen M. Beverley, Robert T. Schimke, Stanford University, Stanford, Cal. 94305; Jeffrey A. Coderre, Daniel V. Santi, University of California, San Francisco, Cal. 94143. Promastigotes of Leishmania tropica, selected for resistance to the antifolate compound methotrexate, contain elevated levels of a bifunctional dihydrofolate reductase-thymidylate synthetase and amplified DNA sequences. Several lines of evidence suggest that the amplified DNA includes the structural gene for the bifunctional enzyme, indicating that the Leishmania have become resistant by the mechanism of specific gene amplification. The amplified sequences are readily detected by electrophoresis and ethidium bromide staining of restriction endonuclease digests of total or nuclear DNA. Summation of the molecular weights of the amplified fragments visualized indicates that an approximately 55 kilobase-pair region(s) of DNA is amplified. Several amplified fragments were isolated and cloned using recombinant DNA methodology; quantitative hybridization of these recombinants with genomic DNAs indicates that these sequences are amplified nearly 100-fold in the resistant line. These data indicate that about 10% of the DNA in the resistant Leishmania consists of amplified DNA.

Reconstruction experiments suggest that amplifications of only 10-fold are detectable; however, the sensitivity varies greatly with the specific restriction endonuclease used and the frequency of recognition sites in the genome. Our results suggest that the prevalence of DNA amplification in drug-resistant Leishmania may be rapidly assessed. Additionally, specific gene amplification provides a convenient approach for the identification and isolation of genes of interest.

0022 A PARASITE PHOSPHOPROTEIN ASSOCIATED WITH THE HOST RED CELL MEMBRANE DURING PLASMODIUM BERGHEI INFECTION, Mark F. Wiser, Patricia A. Wood, John W. Eaton and J.R. Sheppard, Dight Institute, Univ. of Minnesota, Minneapolis, MN 55455.

Red cell plasma membranes isolated from Plasmodium berghei (NYU-2 strain) infected murine red cells display a substantially different pattern of protein phosphorylation than membranes from uninfected cells when examined by SDS-PAGE and autoradiography. When either intact cells are incubated with $^{32}P_i$ (in vivo phosphorylation) or isolated red cell membranes are incubated with γ-$^{32}P_i$-ATP (in vitro phosphorylation), a major new phosphoprotein of 45,000 daltons (pp45) appears exclusively on the host cell membrane. Two-dimensional gel electrophoresis indicates that pp45 is of parasite origin. During subcellular fractionation of parasitized cells, pp45 is found to be preferentially associated with the host red cell membrane rather than the isolated parasites. Extraction of isolated membranes with either 0.5% NP-40 or 0.1 N NaOH reveals that pp45 is insoluble in detergent and only partially extractable with NaOH suggesting that pp45 is tenaciously associated with the host cell membrane. The degree of phosphorylation of pp45 is proportional to the percentage of parasitized cells and is Ca^{2+} independent. The pp45 is phosphorylated at the same amino acid residues in vivo and in vitro indicating pp45 may be physiologically important in the parasite-host relationship.

0023 CHARACTERIZATION OF A PROTEIN KINASE FROM PLASMODIUM BERGHEI, J.R. Sheppard, John W. Eaton and Mark F. Wiser, Dight Institute, Univ. of Minnesota, Minneapolis, MN 55455

Plasmodium berghei infected murine red cells are found to contain increased activity of a protein kinase which is specifically associated with the isolated parasites. Separation of schizont infected cells from ring stage and trophozoite infected cells by density gradient centrifugation in Percoll-Hypaque reveals that the schizonts contain a 4-fold higher activity of this protein kinase than the rings and trophozoites suggesting a role for this enzyme in the development ofthe parasite. The protein kinase was purified 4-fold on DEAE-cellulose and subjected to futher characterization. The Km for ATP is 20 µM while the Km for GTP is 300 µM and the protein substrate preference is phosvitin > casein >> histone (II-A)$_{2+}$. The Mg^{2+} optimum is 10-20 mM and the protein kinase activity is not affected by cAMP, Ca^{2+} or calmodulin. Polyamines stimulate the protein kinase 2-4 fold. Spermine had a negligible effect on the Km of ATP but increased the Vmax 2-4 fold. The flavone, quercetin, inhibits the protein kinase activity in a competitive manner with ATP and has a Ki of 3 µM. Quercetin inhibition and spermine stimulation of the kinase are independent of one another. P. chabaudi also has a very similar protein kinase activity which is stimulated by spermine and inhibited by quercetin. These results indicate that protein phosphorylation catalyzed by a parasite enzyme participates in the growth and development of the malarial parasite.

0024 CALMODULIN IS AN IMPORTANT COMPONENT OF AFRICAN TRYPANOSOMES, Larry Ruben, Charles Egwuagu and Curtis L. Patton, Yale University, New Haven, CT 06510

Studies were initiated to determine whether calmodulin (CaM) was utilized by African trypanosomes in a manner analagous with host tissues to coordinate complex morphological and biochemical life cycle changes. CaM was identified as a major component of particulate and cytosolic fractions obtained from T. brucei brucei. The CaM content varied, however, as organisms progressed from slender to procyclic forms. Trypanosome CaM was characterized on the basis of: mobility on SDS gels; Ca^{+2}-induced conformational changes; CNBr-cleavage fragments; ability to activate bovine brain cyclic nucleotide phosphodiesterase in both a Ca^{+2}-dependent and CaM-dependent manner; ability to activate human RBC (Ca^{+2}-Mg^{+2})ATPase; and inhibition of CaM activity by trifluoperazine and penfluridol. Using these criteria, trypanosome CaM was shown to be distinct from rat RBC or bovine brain CaMs and was most related to CaM isolated from the ciliate protozoan, Tetrahymena. The potential usefulness of CaM as a target for chemotherapy was explored using CaM-binding antipsychotic phenothiazines as probes. Trifluoperazine at low concentrations was cytotoxic to trypanosomes ($LD_{50}$of 15 µM) while the nonCaM-binding derivative, trifluoperazine sulfoxide, exerted minimal effect on cell viability at concentrations of 100 µM. We conclude that trypanosomes contain large quantities of CaM which is distinct from host CaM. Trypanosome CaM has the potential to play an important role in mediating the host-parasite relationship.
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0025 LIFE CYCLE-RELATED DIFFERENCES IN PHOSPHORYLATION OF MEMBRANE PROTEINS IN TWO STRAINS OF TRYPANOSOMA BRUCEI, Patricia E. Mancini and Curtis L. Patton, Yale University, School of Medicine, New Haven, CT 06510.

Phosphorylation of plasma membrane proteins has been examined in T. brucei strains YTat1.1 and 427. Endogenous membrane-associated protein kinase (PK) activity, detected in both strains of the parasite by transfer of ^{32}P label from [γ-^{32}P] ATP to protein, catalyzes the phosphorylation of protein substrates within the membrane as well as the phosphorylation of soluble substrates such as histone. Five membrane polypeptide bands are identified by SDS-PAGE and autoradiographic analysis as the major phosphorylated species, and these are identical in the two species. The phosphopeptides have M_r= 170K, 130K, 100K, 76K, and 55K daltons. The 55Kd band is not VSG since its phosphorylation pattern is the same in both strains despite differences in molecular weight of the VSGs of each strain (YTat1.1= 58Kd; 427=65Kd). Neither soluble nor membrane-associated VSG appears to be a major substrate for PK activity in either strain, and purified VSG does not autophosphorylate. PK activity is observed in the membranes of YTat1.1 bloodstream slender and short stumpy forms, and in slender forms of 427. However, overall phosphate incorporation is lower in stumpy forms. In addition, individual phosphopeptide bands in stumpyform membranes demonstrate less phosphate incorporation than the equivalent band in slender membranes, indicating some developmental control of PK activity and/or accessibility of membrane protein substrates. Neither cyclic AMP nor Ca^{++} affects the phosphorylation patterns of membrane protein substrates qualitatively or quantitatively. (Supported by NIH grant #2 R01 AI 15742)

0026 UNUSUAL SUBCELLULAR DISTRIBUTION OF ENZYMES IN TRYPANOSOMES, LEISHMANIA AND CRITHIDIA Winston E. Gutteridge* Mary J. Davies, David J. Hammond* and Mark B. Taylor, Biological Laboratory, University of Kent, Canterbury, Kent, England.

Initial studies of the distribution of glycolytic enzymes in Trypansoma cruzi showed that the early ones occurred in particulate centrifugal fractions, suggesting a glycosomal origin as in Trypanosoma brucei. This has now been confirmed by analysis of fractions from isopycnic sucrose gradients. Further work has shown that there is also an association of some enzymes of purine and pyrimidine metabolism with the glycosome. In particular, oratate phosphoribosyltransferase and orotidine 5'- phosphate decarboxylase, the last two enzymes of the pyrimidine biosynthetic pathway de novo and hypoxanthine/guanine phosphoribosyltransferase, an enzyme involved in purine base salvage, are all located on or in this organelle. Other workers have demonstrated an association of phospheonolpyruvate carboxykinase with it. It is clear therefore that the subcellular distribution of a number of enzymes from a range of pathways is quite distinct in T. cruzi from that seen in eukaryatic cells generally. A similar situation occurs in other members of the Kinetoplastida.

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0027 ARGININE CATABOLISM BY THE PARASITIC FLAGELLATE TRICHOMONAS VAGINALIS David J. Linstead and Margaret A. Cranshaw, Dept. of Biochemical Parasitology, Wellcome Research Laboratories, Beckenham, Kent, BR 3 3BS. England.

The route of arginine catabolism has been elucidated using whole cells and extracts of Trichomonas vaginalis. The organism has been found to use the arginine deiminase pathway. Arginine is converted to citrulline by arginine deiminase (EC 3.5.3.6.), citrulline is then broken down to ornithine and carbamyl phosphate by a catabolic ornithine carbamoyltransferase (EC 2.1.3.3.)

Carbamyl phosphate is further acted on by carbamate kinase (EC 2.7.2.2.) while ornithine is converted to putrescine by ornithine decarboxylase (EC 4.1.1.17). Each of these activities has been assayed and partially characterised. Arginase, urease, citrulline hydrolase and argino succinate layase appear to be absent from T. vaginalis. A substate level phosphorylation by carbamate kinase is associated with the pathway but the significance of this to the energy balance of the cell in its normal host is at present obscure.

0028 NUCLEOSIDE SYNTHESIZING AND CLEAVING ACTIVITIES IN TRYPANOSOMA CRUZI EXTRACTS, Richard L. Miller and Carol L. Sabourin, Wellcome Research Laboratories, Research Triangle Park, NC 27709

Extracts of Trypanosoma cruzi epimastigotes were found to possess four enzymes capable of cleaving nucleosides. These enzymes have been separated and characterized. Two of the enzymes are hydrolases. Inosine hydrolase (MW 120,000) is specific for 6-oxo-purine containing ribonucleosides. Common product analysis is consistent with a common catalytic site for the cleavage of inosine and guanosine. Deoxyinosine hydrolase (MW 21,000) is specific for 6-oxo-purine deoxyribonucleosides. The other two enzymes are phosphorylases. Adenosine phosphorylase (MW 68,000) requires a 6-amino-substituted purine ribo- or deoxy-ribo- nucleoside for substrate activity. Uridine phosphorylase (MW 54,000), the most abundant nucleoside cleaving enzyme, catalyzes the cleavage of 4-oxo-containing pyrimidine ribo- and deoxyribo- nucleosides. Initial velocity analysis of the synthetic and cleavage reactions for the ribonucleoside and deoxyribonucleoside substrates of the phosphorylases indicate that these reactions proceed via sequential mechanisms. These nucleoside metabolizing activities are similar to those reported for Leishmania (Koszalka, G. W. and Krenitsky, T. A., 1979, JBC 254, 8185-8193) with the exception that T. cruzi possesses a uridine phosphorylase in place of the uridine hydrolase of L. donovani.

0029 CYTOSKELETAL PROTEINS OF TWO PROTOZOAN PARASITES, Joseph D. Schwartzman, University of Virginia Medical Center, Charlottesville, VA 22908.

Actin, myosin and tubulin are nearly ubiquitous constituents of eukaryotes. We have examined their distribution by immunofluorescence in two intracellular protozoa, Toxoplasma gondii and Leishmania donovani because they may play a role in either parasite motility or host cell penetration. T. gondii, which is obligately intracellular, has myosin localized at its anterior pole, in the region of the apical organelles that are thought to function in the process of host cell entry. Suprisingly, two different antisera against actin, as well as the mycotoxin phallicidin which specifically binds to f-actin, failed to show a similar distribution of actin in T. gondii. L. donovani, shows no specific localization of either actin or myosin, in either the extracellular promastigote or intracellular amastigote form. Immunofluorescent staining for tubulin, utilizing rabbit antisera and monoclonal antibodies specific for β-tubulin, showed specific localization of tubulin in the anterior half of T. gondii. No subpellicular microtubules could be detected. One of seven monoclonal anti β-tubulin antibodies failed to stain the cytoplasmic tubulin of T. gondii, while the other six showed closely similar patterns. L. donovani promastigotes showed prominent staining of flagellae as well as membrane fluorescence correlated with abundant subpellicular microtubules. Amastigotes showed only a subpellicular pattern of anti-tubulin fluorescence, and no flagellar staining. One of the monoclonal antibodies failed to stain L. donovani flagellae. The ability to distinguish different populations of tubulin in L. donovani may be useful in studying the process of conversion between intracellular and extracellular forms of this parasite.

0030 ISOLATION AND CHARACTERIZATION OF FORMYCIN B-RESISTANT PROMASTIGOTES OF LEISHMANIA DONOVANI, Buddy Ullman, Univ. of KY, Lexington, KY 40536

From a mutagenized wild-type (DI-700) population of Leishmania donovani promastigotes, a series of clones was isolated by virtue of growth resistance to 5 μM formycin B. A single clone, Fb-D5 was remutagenized, and two colonies, Fb-D5-A3 and Fb-D5-A4, resistant to 50 μM and 20 μM formycin b, respectively, were characterized further. In comparative growth rate experiments, the Fb-D5 cell line was 100-fold less sensitive to formycin b mediated growth inhibition than the DI-700 parent, whereas the Fb-D5-A3 and Fb-D5-A4 clones were 20,000-fold less sensitive. The formycin b-resistant cells were also cross-resistant to formycin a and allopurinol riboside, but as sensitive as DI-700 to a spectrum of other cytotoxic nucleoside analogs. All mutant cells could salvage purine bases and nucleosides normally. However, in comparative uptake experiments, all the formycin b-resistant clones were incapable of accumulating intracellular phosphorylated [^{3}H]-formycin b metabolites. The biochemical nature of the genetic lesion in the formycin b-resistant cells is currently being elucidated.

0031 EFFECTS OF ALLOPURINOL UPON RNA METABOLISM IN LEISHMANIA DONOVANI, Douglas L. Looker, Randolph L. Berens, and J. Joseph Marr, University of Colorado Health Sciences Center, Division of Infectious Diseases, Denver, Colorado 80262

Allopurinol (4 hydroxypyrazolo-(3,4-d) pyrimidine, HPP) a purine analogue, is converted to allopurinol ribonucleoside-5'-monophosphate (HPPR-MP), by several parasitic hemoflagellates. In Leishmania donovani, HPPR-MP inhibits the purine interconversion enzymes adenylosuccinate synthetase and GMP reductase, suggesting that HPP treatment results in a state of purine starvation. However, purine nucleotide pool levels are not reduced. Present studies indicate that HPP treatment of L. donovani promastigotes also affects RNA and protein synthesis. Protein synthesis is reduced 4-6 hours after addition of the drug. By 24 hours RNA content is reduced 50% due to degradation of all RNA species. However, incorporation of radiolabeled uracil into RNA is increased as a result of increased exchange of RNA-derived uracil with the external medium. When RNA is prelabeled with ^{14}C-hypoxanthine, subsequent drug treatment results in a 90% increase in the specific activity of ATP, indicating that purines derived from RNA degradation are used to resupply the soluble purine nucleotide pools.

0032 RESISTANCE OF G-6-PD DEFICIENT ERYTHROCYTES TO PLASMODIUM FALCIPARUM UNDER CONDITIONS OF DIAMIDE-INDUCED OXIDANT STRESS, Jacqueline Miller, Jacob Golenser, Dan T. Spira*, Nechama S. Kosower**, *Hebrew University, Jerusalem, **Tel Aviv University.

Pretreatment with the thiol-oxidizing agent diamide reduced the capacity of G-6-PD deficient but not of normal erythrocytes to support the in vitro growth of P. falciparum. Diamide oxidized reduced glutathione (GSH) to glutathione disulphide (GSSG), diminished membrane SH and resulted in the formation of high molecular weight protein in the red cell membrane. Under culture conditions the normal erythrocyte reversed these changes but the G-6-PD deficient cell did not. Inhibition of P. falciparum development may be due to a number of factors. For example, invasion of the merozoite may be prevented by loss of deformability of the red blood cell or by unavailability of surface cell receptors. Protein synthesis may be inhibited by GSSG and there may be alterations in transport and enzyme functions of the membrane.

Diamide also had a destructive effect on parasitized erythrocytes: mature parasites were more sensitive than ring forms and parasites in G-6-PD deficient cells more sensitive than those in normal cells. There may be two mechanisms whereby diamide inhibits P. falciparum development: (1) by causing alterations in G-6-PD deficient erythrocytes and (2) by direct action on the parasite. This may explain the relative resistance in vivo of G-6-PD deficient individuals to falciparum malaria.

0033 SPECIFIC CHEMICAL INHIBITION OF DIFFERENTIATION IN TRYPANOSOMA CRUZI, Gwyn T. Williams, St. George's Hospital Medical School, London SW17 ORE, U.K.

Morphological differentiation is an obligatory part of the life cycle of Trypanosoma cruzi both in the mammalian host and in the insect vector. In vitro, amastigotes co-cultivated with mammalian cells at 37°C differentiate to trypomastigotes and epimastigotes when removed from the mammalian cells and transferred to Warren's medium at 27°C. This differentiation is blocked by several compounds, such as 5-methylnicotinamide and 3-methoxybenzamide, which are known to inhibit ADP-ribosyl Transferase (poly ADP-ribose polymerase). The block occurs early in the differentiation process and can be reversed by removal of the inhibitor soon after transfer to 27°C. The effect of the inhibitors is specific for differentiation as proliferation is not significantly affected under the same conditions. Intracellular differentiation of amastigotes to trypomastigotes is also inhibited. Since this process is an absolute requirement in the infection cycle, it may be possible to exploit such inhibition of differentiation in the chemotherapy of Chagas' disease and other protozoal diseases.

Inhibitors of ADP-ribosyl Transferase also block differentiation in several vertebrate systems (Farzaneh, Zalin, Brill and Shall, (1982), Nature, in the press, Johnstone and Williams (1982), Nature in the press). The enzyme may therefore play a fundamental role in the differentiation of both unicellular and multicellular eukaryotes.

0034 PURINE AND PYRIMIDINE METABOLISMS IN TRITRICHOMONAS FOETUS, Ching C. Wang, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143.

Studies on precursor incorporation into purine and pyrimidine nucleotide pools in T. foetus indicate that the parasite is incapable of de novo synthesis of both purine and pyrimidine nucleotides. Its growth is dependent on salvage of purines, pyrimidines or their nucleosides. HPLC analyses show that hypoxanthine, xanthine and guanine are converted to the corresponding nucleotides by phosphoribosyl transferase activities in T. foetus. Adenine is incorporated first into IMP; no adenine phosphoribosyl transferase activity is found in T. foetus. Other purine salvage enzymes identified in T. foetus are adenosine kinase and a pelletable enzyme guanosine phosphotransferase.

In pyrimidine salvage, the parasite has uracil phosphoribosyl transferase which enables incorporation of uracil into UMP as one major pyrimidine salvage pathway. There is no incorporation of orotate, cytosine or thymine. Uridine is incorporated through a phosphotransferase or a prior conversion to uracil by phosphorylase. There is no thymidine kinase, thymidylate synthetase or dihydrofolate reductase detectable; deoxyuridine cannot be incorporated into DNA, and the organism grows normally in millimolar concentrations of methotrexate, pyrimethamine, trimethoprim and hydroxyurea. The parasite does incorporate thymidine into TMP, however, through a pellet enzyme thymidine phosphotransferase, which can be inhibited by guanosine. This thymidine phosphotransferase activity provides the only apparent TMP supply for T. foetus, and could be a potential target for chemotherapeutic treatment of the parasite. (Supported by NIH Grant AI-19391)

0035 STUDIES OF HOST-PARASITE INTERACTION IDENTIFY BIOCHEMICAL PATHWAYS FOR CHEMOTHERAPY TARGETS, June Whaun, Nesbitt Brown and H.Kyle Webster, Walter Reed Army Inst. Research Washington, D.C. 20012

We have examined in vitro some biochemical mechanisms of host-parasite interaction with a view toward the rational design of targetted chemotherapeutic agents. Using cultures of P. falciparum-infected human red cells and examination of intermediates with HPLC, we have studied pathways of purine salvage synthesis as well as polyamine metabolism. With regard to purine salvage pathways, while both the parasite and the host cell utilize adenylates, only the parasite (which makes DNA) requires guanylates. Applying this knowledge to chemotherapy, we selected bredinin, an imidazole antibiotic, because of its known selective action on guanylate metabolism in other systems. We were able to show inhibition of the guanylate pathway resulted in inhibition of parasite growth. Furthermore, the environment modified the purine metabolism of the host, inducing an increase in red cell ATP in parasitized cells. Application of these principles to polyamine metabolism yielded similar results. Polyamine metabolism, de novo in both host and parasite, was less easily interrupted as both possessed compensatory mechanisms for inhibition at a single step of biosynthesis. Disturbances of polyamine metabolic pathways induced by inhibition of polyamine biosynthesis by d,l-α-difluoromethylornithine and/or methylglyoxalbis(guanylhydrazone) were associated with poor parasite growth and altered levels of polyamines.

0036 MALARIAL AND ERYTHROCYTE PURINE SALVAGE ENZYMES, Christina M. Schimandle and Irwin W. Sherman, University of California, Riverside, CA 92521

Comparative studies of adenosine deaminase (ADA) and hypoxanthine phosphoribosyltransferase (HPRT) from the malarial parasite, Plasmodium lophurae, and its host cell, the duck (Anas domesticus) erythrocyte have shown many similarities as well as several intriguing differences. The purified ADAs had similar molecular weights, broad pH optima (pH6.8-8.0), and similar K_m values with adenosine. In addition the stabilities of the ADAs when stored as crude lysates were similar. More interesting than the similarities were the differences. The parasite ADA exhibited an average 60 fold higher specific activity in the crude lysate than the erythrocyte ADA. The parasite ADA had a pI of 5.4, and the erythrocyte ADA had a pI of 4.7. Inhibitor studies with ara-A and coformycin showed very slight differences in the K_I values with the ADAs, but EHNA completely inhibited erythrocyte ADA at 1.3μM, but the parasite ADA was not inhibited at 422μM. A further difference was demonstrated by the inability of the parasite ADA to bind to an adenosine affinity column which still bound the duck erythrocyte ADA.

Preliminary studies with HPRT from the erythrocyte and parasite also showed similarities and differences. Both enzymes showed a similar pH optimum at pH 9.5. However, again the parasite HPRT demonstrated a higher specific activity as a crude lysate. The pI's were also different. The parasite HPRT had a pI of 7.5, and the erythrocyte HPRT had a pI of 8.0. In contrast to HPRT, guanine phosphoribosyltransferase (GPRT) exhibited a higher specific activity in the duck erythrocyte lysate than the parasite lysate. The duck erythrocyte lysate exhibited similar specific activities for both HPRT and GPRT.

0037 LACTOSAMINATED BOVINE SERUM ALBUMIN AS A CARRIER FOR PRIMAQUINE IN THE CHEMOTHERAPY OF MALARIA, Jan Hofsteenge, Anne M. Capuano, Rita Altszuler, and Stanford Moore, The Rockefeller University, NY, NY 10021 and New York University, NY, NY 10010

Primaquine is therapeutically active against the exoerythrocytic stages of several species of Plasmodia parasites, but its high host-toxicity, especially its hemolytic effect, poses a serious problem. Coupling this drug to carriers which are specifically taken up by hepatocytes, e.g. lactosaminated proteins (Wilson, G.(1978) J.Biol.Chem. 253, 2070-2072), might result in an increased therapeutic efficacy and/or a reduced host-toxicity.

We synthesized a sulfhydryl-containing primaquine derivative, (cysteinyl-primaquine),which could readily be linked via an S-S bridge to lactosaminated bovine serum albumin that contained propionyldithiopyridyl groups (12-13 mole of drug/mole of lactosaminated BSA).

The causal prophylactic activity (CPD_{50}) of this protein-drug complex was determined in mice infected with Plasmodium berghei. The CPD_{50}-value was found to be about 2.4 times lower than that of free primaquine when administered by the i.v. route (6.0 mg base/kg and 14.3 mg base/kg, respectively). Moreover, the acute lethal toxicity of the protein-coupled drug was very low. The highest dose that could be administered, 85 mg base/kg, (higher doses were impractical because of the limited solubility of the protein-drug complex) did not kill any of the treated mice. In contrast, the toxicity of free primaquine was high in mice: LD_{50}=15.7 mg base/kg and LD_{10}=13.4 mg base/kg. Thus, at least a thirteen fold increase in the therapeutic index (LD_{50}/CPD_{50}) was obtained by coupling primaquine to a hepatocyte-specific carrier.

This work was supported in part by NIH grant # 5-25323.

0038 LIGHT-ENHANCED FREE RADICAL FORMATION AND TRYPANOCIDAL ACTION OF GENTIAN VIOLET (CRYSTAL VIOLET), Roberto Docampo*, Silvia N.J. Moreno*, and Ronald P. Mason°, *Universidad de Buenos Aires, Buenos Aires, Argentina, °LEB, National Institute of Environmental Health Sciences, Research Triangle Park, N.C. 27709

Transmission of Chagas' disease by transfusion of blood in endemic and nonendemic areas has often been reported. Surveys among candidates for blood donors revealed a high percentage of positive serological tests in different countries of Latin America, and gentian violet, a triarylmethane dye, is widely used by blood banks in attempts to eliminate such transmission. We have now found that gentian violet undergoes a one-electron reduction by Trypanosoma cruzi intact cells to produce a carbon-centered free radical as demonstrated by electron spin resonance. The formation of this species, and the trypanocidal action of gentian violet are enhanced by light. Either NADH or NADPH can served as a source of reducing equivalents for the production of the free radical by T. cruzi homogenates.

0039 GENERATION OF FREE RADICALS FROM METRONIDAZOLE AND OTHER NITROIMIDAZOLES BY TRITRICHOMONAS FOETUS, Silvia N.J. Moreno*, Ronald P. Mason°, and Roberto Docampo*, *Universidad de Buenos Aires, Buenos Aires, Argentina, °LEB, National Institute of Environmental Health Sciences, Research Triangle Park, N.C. 27709

Metronidazole, ronidazole, secnidazole, benznidazole and misonidazole are reduced by Tritrichomonas foetus intact cells to nitro anion radicals as indicated by electron spin resonance spectroscopy. This activity appears to be related to the cellular content of reducing substrates, since nitro anion radical formation is stimulated in the presence of glucose and pyruvate. The nitro anion radicals could not be detected under aerobic conditions. Anaerobic homogenates of T. foetus also reduce metronidazole to the nitro anion radical when pyruvate, NADH or NADPH are added as the ultimate source of reducing equivalents. This free radical formation may be the basic cause of nitroimidazole toxicity in Trichomonads.

Identification, Characterization, and Some Molecular Genetics of Parasite Antigens

0040 A COMMON PROTEIN ON THE SURFACE OF MALARIAL MEROZOITES, Anthony A. Holder and Robert R. Freeman, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, U.K.

High molecular weight proteins synthesised during schizogeny have been shown to be expressed on the surface of malarial merozoites. In Plasmodium yoelii a 230,000 MW protein is processed to a series of lower molecular weight fragments, in particular a 90,000 MW polypeptide, which are recognised by a monoclonal antibody. The antibody also reacts with the surface of free merozoites in suspension. Purified 230,000 MW protein and its fragments can be used to immunise mice and prevent subsequent challenge infection with the homologous parasite. In P. falciparum a 195,000 MW protein is made from the onset of schizogeny, as determined with synchronised cultures. At the time of merozoite release and reinvasion of new red cells, this protein is similarly processed, in particular a 83,000 MW polypeptide is a major product. It is proposed that these proteins are analogous and that the processing is a specific mechanism which may be involved in the function of the protein.

0041 MURINE HUMORAL RESPONSES TO PLASMODIAL INFECTION, Carole A. Long, Thomas M. Daly, William R. Majarian, Elizabeth J. Hoffmann, Robert K. Spees, Akhil B. Vaidya, and William P. Weidanz, Hahnemann University Medical College, Philadelphia, PA 19102

Immunity to malarial infection is a complex phenomenon in which a number of immunologic and physiologic mechanisms interact to determine the outcome of infection. One of the most significant host reactions is the humoral immune response, since there is evidence that antibodies play a role in protection against some species of Plasmodia. We have employed a number of techniques to analyze individual antigen-antibody reacting systems, including immunoprecipitation of metabolically labeled plasmodial antigens followed by SDS-PAGE, the preparation of monoclonal antibodies, and the analysis of humoral responses of susceptible and resistant mouse strains. Immunoprecipitation and SDS-PAGE have revealed that a minimum of 30 polypeptides are specifically recognized by the immune murine host. Since antibodies to protective antigens may be late-arising, specific for the late intraerythrocytic stages of infection, and specific for the infecting species of Plasmodia, we have characterized the humoral response to P. yoelii infection with regard to these parameters. Individual antigens have been further analyzed, using hybridomas secreting monoclonal antibodies to P. yoelii (17X) and P. chabaudi. Finally, the kinetics of the humoral response as well as other physiologic parameters have been compared in a number of mouse strains displaying different patterns of susceptibility to infection with cloned P. yoelii.

0042 MOLECULAR BIOLOGICAL STUDIES ON MURINE MALARIAL PARASITES, Akhil B. Vaidya, William Schleif, Prema Arasu, and Carole A. Long, Hahnemann University Medical College, Philadelphia, PA 19102

We are studying murine malarial parasites, using cell-free translation of mRNAs and recombinant DNA techniques. A majority of the mRNAs for Plasmodium yoelii antigens (i.e., those proteins recognized by hyperimmune sera) appear to be polyadenylated and range in size from 15 to 28S. Some of the mRNAs seem to lack poly A or to have such short poly A stretches that they do not bind oligo(dT)-cellulose. Most of the polypeptides coded by such non-poly A^+ RNAs appear to have different molecular weights than those coded by poly A^+ RNAs, indicating that the non-poly A^+ mRNAs are not necessarily the products of partially degraded poly A^+ mRNAs. Two different monoclonal antibodies were used to immunoprecipitate polypeptides coded by sucrose gradient-fractionated mRNAs in cell-free translation reactions. One of the monoclonals precipitated a 47 kd protein; the mRNA coding for this protein was polyadenylated and had a sedimentation coefficient of approximately 19S. Another monoclonal precipitated a series of polypeptides ranging in molecular weight from 230 kd to 73 kd. Surprisingly, the mRNA(s) coding these polypeptides was present in the non-polyadenylated fraction and sedimented with anomalous coefficient of 22-24S. In addition to the cell-free translation studies, we are also investigating the organization of plasmodial genes and the degree of conservation of some of the highly conserved eukaryotic genes (e.g., actin). For these investigations, we have derived libraries of plasmodial genes cloned in E. coli.

0043 ONE STEP PROCEDURE FOR SEPARATION OF ONE OF THE TARGET ANTIGENS OF TRANSMISSION BLOCKING IMMUNITY IN PLASMODIUM GALLINACEUM, Nirbhay Kumar and Richard Carter, Malaria Section, LPD,NIAID,NIH, Bethesda, Maryland 20205.

The basic structural feature of integral membrane proteins is a hydrophobic surface which anchors it to the lipid bilayer and can only be solubilized with the help of detergents. Recently Triton X-114, a detergent hydrophobic enough to be soluble in water has been shown to be useful in separating membrane proteins. This separation is based on a microscopic phase separation of the detergent from the aqueous phase at the cloud point (≥20°C).

In gametes and zygotes of the chicken malaria parasite, P. gallinaceum, two surface proteins have been identified as the potential target antigens of transmission-blocking immunity. Using Triton X-114, we have been able to separate them from each other in a single step. In addition, only 2 or 3 proteins out of a total of 14-16 solubilized from zygotes, were found to show strong hydrophobic interaction with the detergent and therefore resulting in effective purification. Details of these studies on surface labelled, biosynthetically labelled proteins from male gametes, zygotes and ookinetes of P. gallinaceum and their reconstitution into phospholipid vesicles will be presented.

0044 A PROTECTIVE M_r 74 000 PLASMODIUM KNOWLESI ANTIGEN IN THE MEMBRANES OF SCHIZONT-INFECTED RHESUS ERYTHROCYTES. Rupert Schmidt-Ullrich, Tufts-New England Medical Center, Radiobiology Division, Boston, MA 02111.

The protection of rhesus monkeys against P. knowlesi (P.k.) infections positively correlates with antibodies against a M_r74K glycoprotein that is produced by the parasite and expressed on the surface of schizont-infected red blood cells (SI-RBC). The protective nature of the M_r74K was confirmed by vaccination of six rhesus monkeys. Four monkeys received four injections each of the highly purified membrane antigen in Freund's complete adjuvant. Two monkeys received adjuvant only. Ten days after the last antigen injection the animals were challenged with 10^4 viable P.k. schizonts. While the two control monkeys developed fatal parasitemias within six days all vaccinated animals were partially protected as indicated by a delayed onset of patent parasitemias and self-cure at maximum parasitemias between day 7-12. Immunochemical analyses of the sera of vaccinated monkeys revealed that the M_r74K protein was antigenically related to P.k. components with M_rs near 230K, 140K and 102K. The homology between the M_r230K and M_r74K proteins was confirmed by comparative peptide mapping and degradation after oxidative denaturation of the M_r230K into M_r74K and M_r65-70K components. Five of 15 (iodinatable) peptides are labeled by lactoperoxidase-catalyzed surface iodination indicating that a portion of the M_r74K protein is exposed on the surface membrane of SI-RBC. At least one of these 5 peptides represents a glycopeptide as indicated by metabolic labeling with ^{14}C-glucosamine. As the anti-M_r74K antibodies react with a component of similar M_r of P. falciparum-infected monkey RBCs the interspecies nature of this antigen is currently being explored.

Supported by grants from World Health Organization and Rockefeller Foundation.

0045 CLONING OF PRIMATE MALARIA ANTIGENS IN E. COLI,Francine Perler, Rupert Schmidt-Ullrich, Charles Card, James Lynch, Bo-Quin Qiang, New England Biolabs, Inc., Beverly, Ma 01915 and Tufts New England Medical Center, Boston, Ma 02111

A variety of approaches have established that the erythrocytic stages of Plasmodium modify the membranes of their host cells. Previous studies have established that immunization with singlemalaria proteins results in varying degrees of protection from further infection. We report the identification of cDNA clones encoding a 100,000 MW P. knowlesi antigen. P.knowlesi infected Rhesus erythrocytes were isolated at the late schizont stage. mRNA was purified and translated in vitro using the rabbit reticulocyte system. Anti-P. knowlesi immune sera precipitated in vitro translated proteins of 74K, 100K, and 140K apparent MW. This sera precipitates proteins of the same MW from metabolically labeled material. Therefore, our immune sera recognize the nascent, unmodified proteins. cDNA was synthesized from poly(A) mRNA and inserted into the Pst I site of pBR322 by GC tailing. mRNA was size separated on a methyl-mercury agarose gel. An mRNA fraction encoding only the 100K MW protein was used to synthesize a radioactive single-stranded cDNA which was in turn used to screen the cDNA library. Two clones that hybridized to this probe were found to hybrid-select mRNA encoding a 100K MW protein which was precipitable by immune sera. The cDNA clones encoding the 100K MW protein by the criteria of hybrid-selection of mRNA followed by immune precipitation of the resultant in vitro synthesized protein, contain enough sequence to code for 20% of the protein. We are currently sequencing these clones and searching for larger clones coding for the remainder of the gene.

0046 NUCLEIC ACID BIOSYNTHESIS IN VIVO AND RNA TRANSLATION IN VITRO FOR PLASMODIUM FALCIPARUM, Christine A. Gritzmacher and Robert T. Reese, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Development of a vaccine against human malaria will depend on understanding more of the basic biology of the parasite at the molecular level. To do this we used synchronized P. falciparum cultures to examine macromolecular synthesis during the intraerythrocytic growth cycle of the parasite which exhibits distinct morphological and physiological stages. Using incorporation of radiolabeled nucleic acid precursor into synchronized cultured parasites, we quantitated synthesis of RNA and DNA. Most RNA synthesis occurs during two periods in the 48-hr growth cycle: the first is during development of trophozoites (12-18 hr) and the second is at the beginning of schizogony (24-30 hr). DNA synthesis is greatest during late schizogony (30-36 hr). A single species of DNA is made with density indicative of low (19%) GC content. RNA isolated from specific stages of synchronized parasite cultures has been translated in vitro using rabbit-reticulocyte lysates. Both polyadenylated and unfractionated parasite RNA have been translated successfully in this cell-free system. When translation products are separated by SDS-polyacrylamide gel electrophoresis, patterns of malaria-specific proteins are produced which correlate with gel patterns of proteins produced by cultured P. falciparum. Some of the in vitro translated proteins are specific to the growth stage of the parasite from which the RNA was isolated. Many of the in vitro translation products are precipitated by immune monkey serum indicating that antigenic proteins can be synthesized from purified parasite RNA. In vitro translation and immunoprecipitation will be used as a functional assay to screen cDNA libraries for antigen-specific clones.

0047 I.G. Wright, B.V. Goodger and D.F. Mahoney, CSIRO, Queensland 4068 Australia

Immuno absorbance with mouse monoclonal antibodies was used to purify a single protein from a crude B. bovis bovine red cell mixture. This moderately small protein was found to be a single chain after reduction with M.E. and S.D.S. electrophoresis. Using Western Transfer Analysis a single protein band was demonstrated with a series of unrelated bovine anti-B. bovis sera. Protection of the same magnitude as that induced by a crude B. bovis-red cell extract was demonstrated with this protein when susceptible vaccinated cattle were challenged with virulent B. bovis organisms.

0048 DNA SEQUENCE ANALYSIS OF THE α- AND β-TUBULINS OF TRYPANOSOMA BRUCEI BRUCEI, Susan Sather, Richard Nelson, and Nina Agabian, University of Washington, Seattle, WA 98195

The α- and β-tubulin genes of T. brucei are linked in a tandem repeat of 14-17 members. The unit length of the majority of repeated α- and β-tubulin genes is 3.7 kb; approximately 2.9 kb are coding sequence leaving approximately 0.4 kb for each intergenic region. A clone containing one copy of a genomic 3.7 kb repeat unit has been partially sequenced in the coding regions for α- and β-tubulin and in the intergenic regions. Restriction mapping reveals microheterogeneity among tubulin genes within the repeated unit. One such β-tubulin cDNA clone transcribed from a different β-tubulin gene than that represented in the genomic clone, has also been partially sequenced. Partial amino acid sequences predicted from these various clones show extensive homology with known chicken and mammalian tubulins. These partial sequences reveal 90% and 80% homology between the respective T. brucei and chicken α- and β-tubulin, however, less homology is present at the nucleotide level. Strong codon preferences were found in both α- and β-tubulins; these codon preferences are strikingly different from those determined for T. brucei variant antigen genes (Parsons et al., submitted). The intergenic regions between alternating α and β genes are relatively small. Since Northern analysis indicates that each gene can function as a transcriptional unit, these regions should contain transcriptional control signals. Sequences which may function as promoters have been identified, and comparisons between 5' sequences flanking both the α- and β-tubulin genes will be presented. Studies supported by Rockefeller Foundation, NIH Grant AI-17309, NSF Grant PCM 80-21551 and a Training Grant NIH GM 07270 to N.A.

0049 GENOMIC ORGANIZATION OF VARIANT SURFACE GLYCOPROTEIN GENES IN T. BRUCEI PROCYCLIC CULTURE FORMS, Marilyn Parsons, Richard Nelson, and Nina Agabian, University of Washington, Seattle, WA 98195, and Kenneth Stuart, Issaquah Health Research Institute, Issaquah, WA. 98027.

The bloodstream forms of African trypanosomes possess a coat of variant surface glycoprotein (VSG), which is responsible for the antigenic variation that occurs during relapse infections in the mammalian host. The expression of a VSG, i.e. antigen, can be accompanied by the duplication of the gene encoding that VSG. The expression linked copy, which is inserted at a novel site in the trypanosome genome, is transcriptionally active. Procyclic culture forms (PCFs) do not have a surface coat nor do they possess detectable VSG mRNA. In order to examine the role of VSG gene rearrangements and transcriptional activation, we have established seven T. brucei populations of known variant antigen type (VAT) in culture as PCFs. Comparison of the genomic organization of several VSG genes in bloodstream and PCF populations demonstrated that the VSG genes do not undergo extensive reorganization during conversion to PCF. The contexts of VSG genes in the PCF populations closely resembles those of the bloodstream VAT from which they were derived, but differ between PCFs of different VAT origin. In addition, genomic Southern analyses showed that the expression linked copy of at least one VAT was retained after conversion to PCF. This finding indicates that existing expression linked copies are not always transcriptionally active and suggests that an additional mode of transcriptional regulation functions during at least some stage of the trypanosome life cycle. Supported in part by funds from the Rockefeller Foundation and WHO and Grants NIH AI-17309 to N.A. and NIH AI 17373 and DAMD 17-92-2016 to K.S.

0050 CONSERVATION OF T. B. GAMBIENSE VARIANT SURFACE ANTIGEN GENES. M.E. Selkirk, G.R. Newport, M. Milhausen and N. Agabian, University of Washington, Seattle, WA 98195, and A.R. Gray and J.J. Doyle, ILRAD, P.O. Box 20709, Nairobi.

Early serological studies indicated that stocks of T.b. gambiense isolated from different areas of Nigeria were closely related and produced many variant surface antigens (VSA) in common (Gray, 1972). This suggested a restricted repertoire of VSA relative to that of T.b. brucei. We are examining the VSG repertoire of trypanosome stocks at the genomic level and have constructed cDNA libraries for T.b. gambiense TREU 1256 (serotype antigen P1), 1257 (serotype antigen L2), 1285 (serotype antigen U1) and 1288 (serotype antigen U2). These serotypes, although originally isolated in Nigeria, appear in stocks from areas as widely separated as Senegal and Uganda, and serotypes U1 and L2 have been interpreted as representing basic strain antigens (Gray, 1975; Jones, et al. 1981). We have isolated cDNA clones coding for the VSA of serotypes U2 and P1. Southern analysis of stocks of T.b. brucei, T.b. gambiense, T.b. rhodesiense and T. equinum indicates that these genes are extremely well conserved within the T. brucei group. No uniform arrangement of either U2 or P1 genes was observed among the T.b. gambiense stocks, although similarities were observed with some stocks both within and between the T.b. gambiense and T.b. brucei subgroups. We are extending these studies to a wider range of isolates, and by examination of VSA gene arrangements with regard to their geographical origin, hope to gain an insight into the epidemiology of African trypanosomiasis. Supported in part by funds from the Rockefeller Foundation and NSF Grant PCM 80-21551 to N.A.

0051 NOVEL COMBINATION OF EXPRESSION LINKED AND EXPRESSION INDEPENDENT GENOMIC REARRANGEMENTS IN VARIABLE SURFACE GLYCOPROTEIN (VSG) GENE FAMILIES OF T.B. BRUCEI. Richard Nelson, Marilyn Parsons, George Newport, Kenneth Stuart* and Nina Agabian. University of Washington, Seattle, WA 98195;*Issaquah Health Research Inst., Issaquah, WA 98027.

The IsTat serodeme of T.b. brucei consists of variant antigen types (VATs) sequentially isolated from a single deermouse. cDNA clones specific for seven early VATs were used in Southerns to examine genomic arrangements of VSG gene families in each VAT DNA. IsTat 1.A, 1.5, and 1.11 VSG gene families show evidence of a duplicative transposition in expressing VATs. These expression linked copies (ELCs) are absent in VAT populations arising later in infection. Such rearrangements therefore appear to be associated with transcriptional activation of the VSG gene. Both 1.A and 1.5 VSG gene families also contain one member whose genomic context varies in each VAT. Genomic variation of at least one 1.A gene continues throughout chronic infection (180 days) and is thus temporally dissociated from 1.A antigen expression. Thus a single VSG gene family can show both expression linked and expression independent rearrangements. Two members of the 1.1 VSG gene family undergo coordinate variation suggesting either physical linkage or a novel mechanism of VSG intergene communication. The 1.11 VSG gene family is the IsTat prototype of ELC class VSG genes; however, five of the cDNA probes detect rearrangement of their homologous sequences in VAT 1.11 DNA. Our examination of the representative sample of VATs expressed early in a syringe passed infection suggests that genomic variation associated with VSG genes occurs frequently and often independently of homologous gene expression. Supported by funds from the Rockefeller Foundation and WHO and Grants NIH AI 17309 to N.A. and NIH AI 17373 and DAMD 17-92-2016 to K.S.

0052 RELEASE AND PURIFICATION OF TRYPANOSOMA BRUCEI VARIANT SURFACE GLYCOPROTEIN

George A.M. Cross, Rockefeller University, 1230 York Avenue, New York, NY 10021

Conditions affecting the solubilisation of variant surface glycoprotein (VSG) from T.brucei have been investigated. The results obtained have formed the basis for a convenient method of VSG purification which has been in use in our laboratory for 3 years but have so far failed to explain the mechanism of VSG attachment to the trypanosome surface. The possibility that release occurs by proteolytic cleavage of the C-terminal tail has been explored exhaustively and is now almost certainly eliminated by the elucidated structure of the VSG C-terminal carbohydrate attachment (Holder,A.A. Biochem.J. in press). Under some conditions, VSG release appears to be temperature dependent. Osmotic lysis at 0° releases many cytoplasmic proteins but not VSG which may be subsequently solubilised by incubation for 2-3 minutes at pH 8.0 and 40°. Following high speed centrifugation and passage through a column of DEAE-cellulose, isoelectric focusing may be performed for ultimate purity, extending the preparation time to 28 hours. This final step is unnecessary for many purposes. VSG solubilisation is completely inhibited by 1mM Zn^{2+} but not by 2mM Mg^{2+}, Ca^{2+}, Mn^{2+} or by 1mM TLCK or PMSF or by 10mM iodoacetamide, 5% aprotinin, 100ug/ml pepstatin or by 10mM citrate, EDTA or dibromoacetophenone. Release was not stimulated by ionophore A23187 (9ug/ml 10 min 37°, under which conditions motility was severely impaired) or 9% dioxane (90 min 0° or 5 min 37°). The C-terminal structure recently determined suggests an alternative linkage to the plasma membrane which is the subject of current investigation.

This work was performed whilst the author was at the Department of Immunochemistry and Molecular Biology, Wellcome Research Laboratories, Beckenham, Kent, U.K.

0053 THE VARIABLE SURFACE GLYCOPROTEINS OF *T. EQUIPERDUM* ARE PHOSPHORYLATED, T. Baltz, Ch. Giroud, D. Baltz, F. Duvillier, P. Degand, J. Demaille and R. Pautrizel, Lab. Immunol. Biol. Parasit., Université Bordeaux II, 33076 Bordeaux Cedex, France

The phosphoproteins from several *Trypanosoma equiperdum* variants were studied by labelling the parasites *in vivo* with ^{32}P. Phosphoprotein analysis reveals the presence of a 38000 molecular weight phosphoprotein which is absent when live trypanosomes are previously treated with proteinase K under conditions where only the surface coat containing the variable surface glycoprotein (VSG) is removed. This phosphoprotein was subsequently purified and definitively identified as the VSG by the criteria of molecular weight, isoelectric point, immunological specificity and finger print analysis. Based on cyanogen bromide peptide analysis and partial protease cleavage, the phosphorylated site could be localised to within about 6000 daltons of the COOH terminus. Furthermore, a concentration of 1 mole phosphate per mole of purified VSG was determined. The nature of the covalently bound phosphate was investigated in several ways. The phosphate linkage does not involve serine, threonine, tyrosine, aspartate, glutamate of histidine which are usually the sites of peptide phosphorylation. Furthermore, the linkage is resistant to treatment with several enzymes such as alkaline phosphatase, ribonuclease and deoxyribonuclease. Another interesting possibility is that the phosphorylation occurs as for lysosomal enzymes on oligosaccharide residues located in the C terminal region. These results showing that all VSG molecules contain a phosphate group suggest that phosphorylation plays an important role in VSG transfer and function.

0054 ANTIGENIC POLYMORPHISM OF *TRYPANOSOMA CRUZI*, Fernando Plata, Francisco Garcia Pons, and Harvey Eisen, Institut Pasteur, 75015 Paris, France.

The surface antigens of *Trypanosoma cruzi* were studied to determine whether or not *T. cruzi* trypomastigotes showed antigenic polymorphism. Three pathogenic strains of *T. cruzi* were considered: Y, CL and Tehuantepec. The parasites were maintained in the trypomastigote infectious form *in vitro* by infection of the mouse macrophage tumor cell line J774-1, and they were subsequently cloned *in vitro* by limiting dilution. The surface antigens of six to ten clones from each strain were analyzed with rabbit and mouse antisera directed against trypomastigotes of the three original strains and against various clones. The assays used included trypomastigote agglutination, cell surface immunofluorescence and solid-phase radioimmunoassay. Our results indicate a high degree of polymorphism among the trypomastigotes of the three strains, and point to the existence of "private" antigens particular to a specific strain and of "public" antigens shared by trypomastigotes of different strains. In addition, immune precipitation of labeled trypomastigotes followed by SDS-PAGE analysis showed that our sera recognized a varied assortment of antigenic molecules ranging in size from 20 to 200 kD. "Private" antigens ranged from 45 to 70 kD, while the main "public" antigens migrated to positions equivalent to 75kD and 89kD. In spite of persistent efforts to induce antigenic shifts with four different trypomastigote clones, antigenic variation could not be detected. These studies are presently being extended with monoclonal antibodies directed against infectious trypomastigotes.

0055 ANTIGENS DEFINED BY ANTIBODIES ELICITED BY LIVE TRYPOMASTIGOTES OF TRYPANOSOMA CRUZI, Maria Sonia Martins and Leslie Hudson, Department of Immunology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, United Kingdom.

Patients and mice chronically infected with T. cruzi harbour antibodies with lytic activity against live trypomastigotes, detectable by complement mediated lysis (CML), and antibodies reactive in conventional immunofluorescence (IFA). Mice immunized with fixed parasites only show antibodies detectable by IFA. Evidence suggest that the presence of lytic antibodies play a major role in resistance to repeated challenges (Krettli & Brener, 1982).

Mice infected with a sublethal dose of T. cruzi were boosted twice with live trypomastigotes. Sera from these mice, showing high lytic activity, assayed by CML, immunoprecipitated 4 major polypeptides 159, 71, 56, 52 KD (approx. M.W.) from 125-I labelled membranes of T. cruzi organisms solubilized in Renex 30. Significantly, sera from mice immunized with fixed tissue culture trypomastigotes, with no lytic activity but showing high anti parasite titre by immunofluorescence against fixed epimastigotes, precipitated only the major 56 KD (approx M.W.) component from epimastigote labelled membranes.

*Assistant Professor on leave of absence from Department of Parasitology, I.C.B., University of Minas Gerais, BRAZIL. PhD student sponsored by CNPq BRAZIL.

0056 PATTERNS OF SURFACE ANTIGENS IN STRAINS AND CLONES OF TRYPANOSOMA CRUZI. Bianca Zingales[1], Grace Abuin[1], Alvaro J. Romanha[2], Egler Chiari[2] and Colli[1] . 1 - Instituto de Química, U.S.P. São Paulo, Brazil, 2 - Instituto de Ciências Biológicas, U.F.M.G. Belo Horizonte, Brazil.

Epimastigotes, belonging to four distinct zymodemes, were isolated from chagasic patients of Bambuí (M.G. - Brazil). The radioiodinated proteins from the surface of 12 different stocks, analysed by SDS-PAGE, exhibited a complex pattern, with many components common to all strains and clones screened. Sera, obtained from chagasic patients classified accordingly to each zymodeme and from rabbits immunized with either epimastigote plasma membrane vesicles or trypomastigotes from the Y strain, immunoprecipitated a 95K and a 80K antigen of the surface of all T. cruzi strains tested. The latter results indicate that these antigens, probably glycoproteins, must be common to epi and trypomastigotes. Conversely, when surface radioiodinated trypomastigotes of the Y strain were immunoprecipitated with 16 different human chagasic sera from Bambuí, a remarkable identity among patterns was observed, suggesting that the antigenic characteristics of the surface of T. cruzi infective forms are essentially constant.

0057 SURFACE PROTEINS AND ANTIGENS OF TRYPANOSOMA CRUZI, David Lanar and Jerry Manning. U.C. Irvine, Irvine, CA. 92717.

Six stages of T. cruzi: bloodstream trypomastigotes (BSTM), culture-form trypomastigotes (CFTM), amastigotes(AM), staphylomastigotes (SM), epimastigotes (EM), and metacyclic trypomastigotes (MCTM) were analysed by Iodogen catalized surface iodination for surface proteins. Proteins were compared by 1D and 2D PAGE. Furthermore all six stages were analysed by Western Blotting to detect antigens by reaction with serum from mice hyperimmunised against BSTM. Major results show that BSTM, CFTM, and SM contained several iodinatable 92K proteins with pI's between 4.7 and 5.3. By Western Blotting a 92K protein is the major antigen seen on BSTM, CFTM, SM, and AM. No 92K protein could be detected by either Western Blotting or iodination of EM or MCTM. The major surface proteins on these latter two stages were several 72K proteins with pI's between 4.5 and 4.9. The major result of this survey shows that the 92K surface antigen could now be found in large quantities on staphylomastigotes, a dividing form grown in cell free medium which should facilitate the isolation of the mRNA and gene coding for this protein.

0058 THE STRUCTURE AND FUNCTION OF A CELL SURFACE GLYCOPROTEIN FROM TRYPANOSOMA CRUZI, David Snary[1], Michael J. Ferguson[1], Anthony K. Allen[2] and Alan Sher[3], [1]Wellcome Research Laboratories, Beckenham, U.K., [2]Charing Cross Hospital Medical School, London, U.K., [3]N.I.A.I.D., Bethesda, Maryland, U.S.A.

A 72,000 molecular weight phosphoglycoprotein has been purified from T. cruzi by monoclonal antibody affinity chromatography. The phosphoglycoprotein contains 10% phosphate and 47% carbohydrate which comprises mannose, galactose, ribose, xylose, fucose and glucosamine. Two types of carbohydrate side chain are proposed, one a high mannose type linked by glucosamine to asparagine in the polypeptide chain, and the other a pentose rich chain which is linked through xylose and fucose to the polypeptide. All phosphate is linked to the carbohydrate side chains. The glycoprotein is found on the insect stages but not the mammalian stages of T. cruzi and vaccination will protect mice against an insect derived metacyclic challenge but not a blood trypomastigote challenge. The in vitro morphological transformation of epimastigotes to trypomastigotes is inhibited by the monoclonal antibody to the phosphoglycoprotein and it is proposed that this molecule has a role in the control of morphogenesis of T. cruzi, possibly by interaction with specific gut lectins in reduviid bugs.

0059 SPOROZOITE ANTIGENS OF COCCIDIA. Martin H. Wisher, Houghton Poultry Research Station, Huntingdon, Cambs., PE17 2DA, U.K.

Invasion and development of species of Eimeria in the intestinal tract of chickens renders birds immune to subsequent challenge with the same species. The antigens which induce immunity to these economically important protozoa have not hitherto been characterised. In this study, sporozoite stages of the parasite were radioactively labelled and antigens identified after precipitation by antisera obtained from infected animals. Viable sporozoites (excysted in vitro) were purified by metrizamide density gradient centrifugation. Cell surface proteins were radioiodinated using Iodogen coated tubes and the ^{125}I-polypeptides resolved by polyacrylamide gel electrophoresis (PAGE) in the presence of SDS. The ^{125}I-polypeptide profiles of E.tenella, E.maxima and E.acervulina sporozoites (all infective to chickens) and E.nieschulzi sporozoites (infective to rats) were similar. Up to 16 ^{125}I-polypeptides were resolved with molecular weights (M.W.) from 20,000 to > 200,000. After incubating sporozoites with ^{35}S-methionine up to 27 labelled polypeptides could be resolved by SDS-PAGE.

Immunoprecipitates of solubilised radiolabelled sporozoites (E.tenella) precipitated with specific chicken antiserum and rabbit antichicken IgG serum were resolved by SDS-PAGE. At least 7 specifically precipitated ^{125}I-polypeptides from the surface membrane (M.W. 24,000 to 125,000) and 5 ^{35}S-polypeptides (M.W. 35,000 to > 200,000) were identified. Indirect immunofluorescence with the chicken antiserum strongly labelled the surface membrane of sporozoites and other developmental stages. The identification of these antigens is an important step towards the development of a vaccine.

0060 GLYCOCONJUGATES OF LEISHMANIA DONOVANI, S.J. Turco, Clawson, D.R. and Wilkerson, M.A., Univ. of Kentucky Medical Center, Lexington, KY 40536

We have been investigating the structure, biosynthesis, and function of the glycoconjugates of Leishmania donovani. These parasites were labeled with [^{3}H]mannose and subjected to multiple organic solvent extraction. The dolichol-linked oligosaccharide fraction was analyzed by high pressure liquid chromatography and glycosidase digestion. Unlike other eukaryotic cells, the major lipid-derived oligosaccharides obtained from this fraction had a composition of 6 to 7 mannose residues and 2 N-acetylglucosamine residues. Examination of the assembly of the dolichol intermediates as a function of time or temperature never revealed the presence of any glucosylated oligosaccharide intermediate. We also determined that the $Man_7GlcNAc_2$ oligosaccharide is transferred from the lipid carrier to proteins. Another major finding was the extraction of a [^{3}H]mannose-labeled substance we have tentatively identified as a polysaccharide. This material possesses the following characteristics: 1) solubility in alkaline solvents but not acidic solvents; 2) an approximate molecular weight of 40,000 as determined by gel filtration chromatography on a column of Sephadex G-100 assuming no aggregation; 3) lack of sensitivity to pronase, hyaluronidase, or chondroitinase as examined by gel filtration chromatography; 4) lability to mild acid hydrolysis (0.02 N HCl, 20 min, 100°C); 5) the presence of phosphate as determined by sensitivity to alkaline phosphatase and by chromatography on DE52 cellulose; and 6) hydrophobic properties. (Supported in part by NIH Grant No. AM26983)

0061 POLYSACCHARIDES OF TEGUMENTAL SUBFRACTIONS OF THE RAT TAPEWORM, HYMENOLEPIS DIMINUTA. Nancy Robertson and George Cain, University of Iowa, Iowa City, IA, 52242

Chloroform-methanol extracted residues of the 2,500Xg and 30,000Xg pellets and 30,000Xg supernatant, carcal residues, and whole worms of 10 day old Hymenolepis diminuta were analysed for their complex carbohydrates following treatment by proteases, nucleases, and a-amylase. Eggs from 3 mo. old worms were similarly examined. Gas-liquid chromatography of alditol acetate derivatives showed glucose, galactose, and mannose to be the prevalent monosaccharides in all fractions, and glucosamine and galactosamine were also present. Uronic acid was present in the carcass but was absent in the tegumental(2,500Xg pellet) and tegumental vesicle(30,000Xg pellet) fractions. All fractions contained inorganic sulfate but lacked sialic acid. Gel electrophoresis in agarose-polyacrylamide revealed two metachromatic bands when stained with toluidine blue in the 2,500Xg pellet, neither of which could be identified as heparin, heparan sulfate, hyaluronic acid, or dermatan sulfate on the basis of enzyme sensitivities. This was also true of the one band in the 30,000Xg pellet. These bands showed partial sensitivity towards chondroitinase ABC, suggesting the presence of chondroitin/chondroitin sulfate. On the basis of nitrous acid and Flavobacterium heparinase sensitivity, and resistance towards chondroitinase ABC, bovine testicular and Streptomyces hyaluronidases, the carcass contains heparin /heparan sulfate as its sole glycosaminoglycan(s). The failure of extensive treatment of chloroform-methanol extracted whole worms with trypsin and Pronase to eliminate many polypeptides suggest that one of the functions of the tapeworm's complex carbohydrate complement is to protect it from the host's intestinal proteases.Supported by Edna McConnell Clark Foundation.

0062 EVIDENCE FOR MODIFICATION AND TURNOVER OF SURFACE ANTIGENS OF THE PARASITIC NEMATODE TOXOCARA CANIS, R.M.Maizels, M.W.Kennedy, M.Meghji & H.V.Smith, National Institute for Medical Research, Mill Hill, London, and Stobhill General Hospital, Glasgow.

Toxocara canis is a cosmopolitan canine parasite which can infect man, occasionally causing ocular or neurological lesions. The infective larvae can survive for many years in paratenic hosts such as man, giving rise to visceral larva migrans. In vitro these larvae are not only remarkable for their longevity but for their high production of released (ES or Excreted-Secreted) antigens.

We have characterised both surface and ES sets of molecules from T.canis larvae by radioiodination and have previously reported on the homology between antigens of 110,000 and 115,000 daltons from both sources. These antigens, in which carbohydrate determinants are predominant, are rapidly released from the parasite surface under conditions which suggest that continual turnover of surface antigens may be taking place. However, ES glycoproteins of 32,000 and 70,000 do not have exact analogues on the larval surface; rather there is marked heterogeneity of surface components with a number of bands observed between 35-65,000 daltons. This heterogeneity does not appear to be the consequence of degradation artefacts.

Monoclonal antibodies have been raised against the major ES components, and with a panel of such reagents it has been found that antibody against the 70,000 band binds to lower molecular weight species from the surface. Furthermore the lower weight surface molecules can only be found immediately after radiolabelling and not on larvae cultured for 2 d thereafter. Since these are glycoprotein antigens, the possibility is now being tested that serial glycosylation of ES antigens prior to release is taking place on the nematode surface.

Molecular Tools In Classification and Diagnosis

0063 BIOCHEMICAL AND IMMUNOLOGICAL METHODS FOR THE IDENTIFICATION OF TRYPANOSOMATIDS. E.Plessmann Camargo, C.L.Barbieri, D.Mattei, C.Morel, J.D.Lopes, A.Rodrigues and N.Yoshida. Escola Paulista de Medicina. P.O.Box 20342. CEP 04023. São Paulo, Brazil.

Insect trypanosomatids may be used as probes for testing biochemical methods useful in the identification of heteroxenic trypanosomatids, including human pathogens. Methods may be used for genus, species or strain separation.

Genus distinction was achieved by determination of enzymes of the ornithine-arginine metabolism, according to the following scheme:

1-ARGINASE present: *Crithidia, Leptomonas, Leishmania,Endotrypanum;* CITRULLINASE present: *Crithidia, Leptomonas;* ARGININOSUCCINASE present: *Crithidia.*
2-ARGINASE absent: *Herpetomonas, Trypanosoma;* ARGININE DEIMINASE present: *Herpetomonas.*

Species separation among insect trypanosomatids was achieved by 2 methods: fingerprinting of k-DNA digests by restriction endonucleases and SDS-PAGE of radioactively labeled cell surface proteins. Both methods allow species distinction (21 species examined), ignoring intraspecific variation. This does not apply to human pathogens, where fingerprinting of k-DNA digests distinguishes between strains and even clones of *Trypanosoma cruzi.*

Monoclonal antibodies have been raised against *T.cruzi, C.fasciculata* and *H.m.muscarum.* Most monoclonal antibodies recovered cross-react extensively (IIF test) with species of insect trypanosomatids (18 examined) and *T.cruzi.* This indicates widespread occurence of common epitopes. A few antibodies seem to be restricted to one or few species.

0064 CHARACTERIZATION OF ISOLATES AND CLONES OF LEISHMANIA BY ANALYSIS OF KINETOPLAST DNA, Terry Spithill, Raelene Grumont and Graham F. Mitchell, The Walter and Eliza Hall Institute, Melbourne, Australia.

Isolates and clones of Leishmania were characterized by restriction endonuclease fingerprinting and Southern blot hybridization analysis of kinetoplast DNA. The size of the EcoR1-cut unit length minicircle distinguishes certain isolates since L.t.major isolates (LRC-L137, L38, L251) possess minicircles (680bp) smaller than those in isolates causing diffuse cutaneous (LRC-L285, 720bp), visceral (LRC-L52, 790bp) or recidiva (LRC-L32, 840bp) leishmaniasis, respectively. Fine structure kDNA fingerprints with certain endonucleases can further distinguish isolates L137, L38, L285 and L32 showing that these isolates form different schizodemes. High stringency blot hybridization using as a probe kDNA from the virulent clone L137/7/121 revealed extensive kDNA sequence homology among isolates causing cutaneous (L137, L38, L251) or diffuse cutaneous (L285) disease, suggesting that all these isolates are genetically related and that the previously unidentified isolate L285 may be an L.t.major strain. In contrast, the recidiva isolate L.t.tropica L32 has diverged considerably from L137/7/121 since weak kDNA sequence homology was only observed at low stringency. Similar experiments with four clones of L137 differing in virulence in mice show that isolate L137 is heterogeneous and consists of closely related clones that possess kDNAs having the same unit length minicircle size, exhibit extensive DNA sequence homology and can only be distinguished by minor differences in their kDNA fingerprints. The analysis of kDNA from Leishmania is a valuable tool for determining the relatedness of isolates and clones of this parasite.

0065 ANALYSIS OF KINETOPLAST DNA FROM PATHOGENIC LEISHMANIA, John A. Wohlhieter and Peter R. Jackson, Walter Reed Army Institute of Research, Washington, DC 20012

Leishmania identification is important for both patient management and epidemiological studies. We have investigated the possibility of using agarose gel electrophoresis of restriction enzyme digests of mitochondrial (kinetoplast) DNA (kDNA) isolated from various pathogenic Leishmania species as an identification procedure. Comparison of the banding patterns of the kDNA from various species indicate that this kind of analysis is useful to differentiate Leishmania species responsible for different types of disease. It is difficult to quantitate the differences between these banding patterns so we also have used kDNA hybridizations to measure the relationship between various Leishmania. We have found that 32P-labeled kDNA from an L. donovani clone hybridizes to some extent with all the restriction fragments in the kDNA banding patterns obtained from each of visceral isolates tested but not with fragments of kDNA isolated from Leishmania responsible for cutaneous disease. These DNA hybridization procedures have been modified so that homology or relatedness can be measured between a labeled kDNA probe and minute quantities of purified kDNA, whole parasites or infected tissue. A combination of these techniques has been useful to characterize clonal variations of the same species, the relationship between species isolated from different geographical areas and species causing the same or different clinical pathology.

0066 Variability in Kinetoplast and Nuclear DNA Sequences in Different Isolates of Trypanosoma cruzi. A.C.C. Frasch[1,2], D.G. Sanchez[1], S.G. Goijman[1], J.H. Crosa[2] and A.O.M. Stoppani[1]. [1]Catedra de Bioquimica Facultad de Medicina, Buenos Aires, Argentina and [2]Dept. Microbiology and Immunology, Oregon Health Sciences University, Portland, Oregon

The sequence heterogeneity of kinetoplast DNA obtained from different Trypanosoma cruzi isolates provides a useful method for classifying closely related parasites. Mini-circle DNA obtained from the different T. cruzi isolates was cleaved with different restriction endonucleases. The observed cleavage patterns indicated that all the parasites studied were related with four defined conserved regions within the major mini-circle populations in each T. cruzi isolate. Nonetheless the mini-circles possess sufficiently non-conserved regions as to permit the differentiation of several T. cruzi isolates. This method was also useful in differentiating each one of these isolates from another South American trypanosome, T. rangeli. Two Tulahuen stocks originally obtained from the same isolate, one maintained in culture during 10 years (T0) and the other in mice until 1979 and later in culture (T2), also depicted different mini-circle restriction patterns. Variations between these two stocks were not, however, circumscribed to kinetoplast DNA. A cloned restriction endonuclease fragment from T0 genomic DNA containing repetitive sequences was used as a probe in hybridizations with restriction endonuclease digested nuclear DNA from T0 and T2. This clone which recognized repetitive sequences present in a low copy number per genome, hybridized with distinct restriction fragments, some of them being characteristic of each stock. These results, together with the variations in the surface protein pattern of T0 and T2 isolates, suggest that important genetic changes have occurred during subculture of the parasites.

0067 Rapid Identification of Leishmania Species by Specific Hybridization of Kinetoplast DNA in Cutaneous Lesions. D.F. Wirth, D. McMahon-Pratt, N de Araujo Filho,H. Durado, J.R. David. Harvard University, U.S.A. and Instituto de Medicina Tropical de Manuas, Brazil

Kinetoplast DNA (kDNA) was isolated from various species of the protozoic parasite, Leishmania and analyzed by nucleic acid hybridization to detect species related heterogeniety of kDNA. Purified kDNA isolated from L. mexicana and L. braziliensis displayed no homology in nucleic acid hybridization studied. These results confirmed that rapid kDNA sequence change and evolution is occuring in New World species of leishmaniasis and suggested that such isolated kDNA could be used as a specific hybridization probe for rapid identification of Leishmania species in whole organisms. This work further demonstrates that such species specific identificaton is feasible, both on isolated Leishmania promastigotes and more importantly, directly on tissue touch blots derived from cutaneous lesion tissue. Thus, specific hybridization of isolated kDNA provides the basis for a rapid accurate method for the diagnosis of human leishmaniasis directly from infected tissue. In a preliminary study involving seven patients, leishmania organisms were identified in the lesions from all seven patients. kDNA from the L. braziliensis complex hybridized with lesions from four patients, while L. mexicana kDNA hybridized with lesions from three patients.

0068 CLONING AND CHARACTERIZATION OF AN INDIGENOUS PLASMID FROM CHLAMYDIA TRACHOMATIS, Lindy Palmer and Stanley Falkow, Stanford University, Stanford, CA 94305

We are interested in the genetic organization of the obligate intercellular parasite Chlamydia trachomatis. Chlamydial plasmid and chromosomal DNA sequences have been cloned into E. coli plasmid and phage vectors and the expression of chlamydial proteins in E. coli is being investigated by immunological blotting. An indigenous 7Kb plasmid, pCHL1, was isolated from C. trachomatis LGV 434 and characterized at the molecular level. Distribution of homologous plasmids throughout representative serovars of C. trachomatis was examined by DNA hybridization. 13 out of the 15 immunologically defined serovars contain plasmid DNA sequences which are highly conserved while the remaining 2 serovars show either partial or no DNA homology. All of 36 random clinical isoates were found to carry the indigenous plasmid. Plasmid DNA prepared from clinical isolates by a rapid method was digested with several restriction endonucleases and found to contain only minor heterogeneity of restriction sites. Restriction fragments from pCHL1 have been cloned into the M13 vector MP8 to generate single strand specific DNA probes. The sensitivity of DNA hybridization for the direct detection of chlamydial infection in clinical material is being compared to that of other diagnostic techniques.

0069 EFFECTS OF PYRAZOLOPYRIMIDINE RIBONUCLEOSIDES ON TRYPANOSOMA CRUZI. Randolph L. Berens[1], J. Joseph Marr[1], Donald J. Nelson[2], Stephen W. LaFon[2] and Gertrude B. Elion[2], [1]Division of Infectious Diseases, University of Colorado Health Sciences Center, Denver, CO 80262, [2]Burroughs Wellcome Co., Research Triangle Park, NC.

Studies with the culture forms of Trypanosoma cruzi have shown that its sensitivity to pyrazolopyrimidine ribonucleosides is strain specific. Growth experiments have shown that the epimastigotes of both the Columbia and Costa Rica strains of this parasite are not significantly affected by allopurinol ribonucleoside (HPPR), while the Peru and Y strains are (LD50's of >50 µg/ml and 1 µg/ml respectively. Whole cell incubations with ^{14}C-HPPR indicate that the sensitive strains are 10- to 100-fold more efficient than the resistant ones in their ability to sequentially convert HPPR to its ribonucleotide monophosphate and then aminate it to aminopurinol ribonucleotide mono-, di-, and tri-phosphate which is incorporated into the RNA. Similar experiments with another pyrazolopyrimidine ribonucleoside, Formycin B (FOR-B), show that the strains resistant to HPPR are also less sensitive to this drug. The metabolism of FOR-B was found to be similar to HPPR in that it is also converted to it ribonucleotide monophosphate and then aminated to give Formycin A mono-, di- and tri-phosphate and is incorporated into the cells RNA. Further studies, using the sensitive Peru strain, have shown that the addition of HPPR, at a concentration of 10 µg/ml of culture medium, to infected human diploid lung cells results in an apparent eradication of the infection.

0070 IDENTIFICATION OF NEW WORLD LEISHMANIA ISOLATES BY AGAROSE GEL ELECTROPHORESIS AND POLYACRYLAMIDE GEL ISOELECTROFOCUSING

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In a study of more than 100 isolates from 13 states of Brazil and 5 other Latin American countries seven distinct parasitological agents of leishmaniasis were identified.Identification was based on agarose gel electrophoresis and polyacrylamide gel isoelectrofocusing followed staining for activity of six enzymes: Aspartate aminotransferase (E.C.2.6.1.1.);Alanine aminotransferase (E.C.2.6.1.2.); Malate dehydrogenase (E.C.1.1.1.37); Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49); Phosphoglucomutase (E.C.2.7.5.1.); and Glucose phosphate isomerase (E.C.5.3.1.9.). One agent was responsible for viscerotropic infection which by comparison with Old World reference stocks was identified as Leishmania donovani. No evidence was found from enzyme electrophoresis for the existence of a distinct species L.chagasi or subspecies L.d.Chagasi responsible for American visceral leishmaniasis, confirming results of previous studies.
Isolates of L.brasiliensis could be separated into two groups.By comparison with reference strains these groups were identified as L.b.braziliensis and L.b.guyanensis. Stocks and reference strains of L.b.panamensis could not be separated by enzyme electrophoresis from L.b.guyanensis. The remaining isolates could be divided into four groups, which correspond to L.mexicana amazonensis,L.m.mexicana, and two groups, provisionally labeled'L.mexicana' Type III and Type IV, which represent undescribed subspecies of Leishmania. In addition,stocks of L.m.mexicana could be subdivided on the basis of Malate dehydrogenase variants.Isolates from Venezuela labeled L.garnhami and L.m.pifanoi gave enzyme profiles identical to those of L.m.amazonensis.

0071 THE CHARACTERIZATION OF SPECIES AND STAGE SPECIFIC MONOCLONAL ANTIBODIES FOR LEISHMANIA TROPICA. Charles L. Jaffe and Diane McMahon-Pratt, Harvard Medical School, Boston, Ma. 02115

Monoclonal antibodies were raised against membrane enriched preparations of Leishmania tropica major promastigotes. Six monoclonals XLVI 5A5-D4, XLVI 5B8-B3, XLVI 4H12-C2, LXIX 1A5-G1, LXIX 3E10-B7and LXIX 4F11-E8 were produced which reacted specifically with the L.tropica complex. All of the antibodies react with L.tropica major. In addition, clones XLVI 5B8-B3, LXIX 1A5-G1 and LXIX 4F11-E8 also bind to L.tropica minor and clone LXIX 3E10-B7 binds to L.tropica aethiopica. The remaining two clones react with all members of the L.tropica complex and weakly to L.mexicana amazonesis. No crossreactivity was observed with L.donovani, L.braziliensis braziliensis and Trypanosoma cruzi. When the binding of the monoclonals to intact glutaraldehyde-fixed promastigotes and amastigotes of L.tropica major was examined XLVI 5B8-B3, XLVI 4H12-C2 and LXIX 3E10-B7 were found to be promastigote stage specific. The remaining monoclonals reacted with both stages of the parasite. The antigens for three of the monoclonals were identified by radioimmunoprecipitation of solubilized ^{125}I-lactoperoxidase labelled L.tropica major promastigotes. Clone XLVI 5B8-B3 precipitated two protein antigens of 95 and 150 kilodaltons molecular weight. Three proteins of 70, 100 and 200 kilodaltons were recognized by XLVI 5A5-D4 and XLVI 4H12-C2. The ability of XLVI 5A5-D4, XLVI 5B8-B3 and XLVI 4H12-C2 to protect BALB/c female mice against a challenge by L.tropica major promastigotes was also examined.

0072 NUCLEOTIDE SEQUENCES OF PORTIONS OF THE MAXICIRCLE KINETOPLAST DNA FROM LEISHMANIA TARENTOLAE, V. de la Cruz, N. Neckelmann , L. Simpson and A.M. Simpson, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

The nucleotide sequences of portions of the 30 kb maxicircle DNA from Leishmania tarentolae kinetoplast DNA have been determined and examined for open reading frames and for homologies with known mitochondrial structural genes. The two fragments selected for initial sequence analysis were the 6.6 kb EcoRI/BamHI fragment cloned in pBR322 (pLtl20) which contains the presumtive ribosomal genes and the gene for cytochrome b (Simpson, Spithill and Simpson,1982), and the 4.5 kb MspI fragment 3 cloned as two subfragments in pBR322 (pLtl52 and pLtl50) which contains the genes for cytochrome oxidase subunits I and II (Ibid). Sequences were obtained by the dideoxy termination method using M13 subclones generated either by restriction enzymes or by random DNaseI/Mn++ digestion. Mapping of the 3' and 5' ends of individual transcripts is in progress.

This work was supported by grants from the NIH.

0073 SEQUENCE EVOLUTION OF LEISHMANIA AND TRYPANOSOMA MAXICIRCLE DNA, Michael L. Muhich and L. Simpson, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

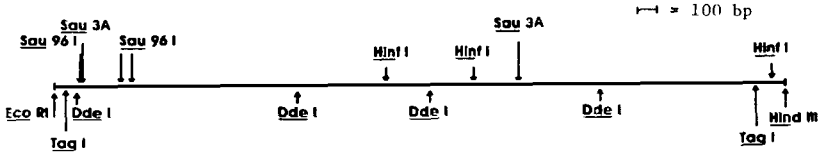
The extent of sequence homology between the 22 kb T. brucei maxicircle and the 30 kb L. tarentolae maxicircle has been evaluated and the organization of the conserved regions determined. Digests of the T. brucei maxicircle were blotted onto filters and probed over a range of hybridization stringencies with labeled fragments of the EcoRI linearized L. tarentolae maxicircle. The organization of conserved sequences was found to be similar in both maxicircle genomes with the highest degreee of sequence conservation localized to the 9S and 12S genes. The greatest sequence diver- was observed within the largest T. brucei HindIII fragment and the largest L.tarentolae MspI fragment. In addition, two smaller less homologous regions were found interspersed within the conserved regions of the T. brucei maxicircle. To explore the potential coding capacity of the T. brucei maxicircle blots of digests were probed with portions of mitochondrial genes from yeast, rat and Zea mays at varying wash stringencies. Hybridization was observed with yeast cytochrome oxidase (COX) I,II and III, cytochrome b and ATPase 6 probes, as well as with rat COX I,II and Zea mays COX II probes.

This work was supported by grants from the NIH.

0074 CHARACTERIZATION OF THE REGION OF THE MAXICIRCLE OF TRYPANOSOMA BRUCEI KINETOPLAST DNA WHICH HYBRIDIZES WITH A MITOCHONDRIAL GENE ENCODING CYTOCHROME OXIDASE SUBUNIT II, Barbara J. B. Johnson, George C. Hill, Kenneth Stuart, and John E. Donelson, Colorado State University, Ft. Collins, CO 80523.

The 20 kbp maxicircles which comprise one portion of the concatenated network of kinetoplast DNA may be the functional equivalent of mitochondrial DNA in other organisms. A 2.8 kbp Taq I fragment of the maxicircle cross-hybridized in Southern blots with a cloned mitochondrial sequence which encodes cytochrome oxidase subunit II (COX II) of Zea mays. The size of this cross-hybridizing Taq I restriction fragment is conserved between T. brucei brucei, T. brucei gambiense, and T. brucei rhodesiense.

The fragment of maxicircle DNA, which putatively contains at least a portion of the structural gene for COX II, was cloned into plasmid pBR325 for further study. A restriction map of the inserted region is shown below. The sequence of this region will be presented.



0075 REVERSIBLE DECATENATION OF KINETOPLAST DNA BY A UNIQUE DNA TOPOISOMERASE ACTIVITY FROM CRITHIDIA FASCICULATA, Joseph Shlomai and Anat Zadok, The Hebrew University, Jerusalem, Israel

An enzymatic activity detected in cell extracts of the hemoflagellate Crithidia fasciculata interlocks kinetoplast DNA minicircles into network forms resembling the natural kinetoplast DNA (kDNA) found in trypanosomatids. Partial purification and characterization of the enzyme responsible for this activity has revealed the existence of an ATP-dependent DNA topoisomerase in Crithidia fasciculata. Catenation of duplex kDNA minicircles is reversible, the equilibrium is affected by the ionic strength, and the reaction is sensitive to high levels of the drug novobiocin. The enzyme capability to discriminate between newly replicated and non-replicated kDNA minicircles as substrates for decatenation suggest that it could fit a role for the "release and reattachment" enzyme postulated by Englund and his colleagues (Englund, 1980) to function in the course of kDNA replication. Other catalytic properties of this enzyme are in support of such a role for the crithidial topoisomerase.

0076 REPLICATION OF THE MAXICIRCLE COMPONENT OF CRITHIDIA KINETOPLAST DNA, Stephen L. Hajduk, Viiu Klein and Paul T. Englund, Johns Hopkins School of Medicine, Baltimore, Md. 21205

The kinetoplast DNA network of Crithidia fasciculata is composed of thousands of interlocked minicircles (2.5 kb) and 20-50 maxicircles (∿34 kb). The replication of the minicircles has been studied in detail and they appear to replicate via Cairns-type intermediates as free molecules. Replication of the maxicircles coincides with that of the minicircles, during a discrete S-phase. Electron microscopy of replicating kDNA networks, isolated on CsCl-propidium diiodide gradients, reveals maxicircle edgeloops which resemble rolling circles. In isolated mitochondria we have identified a population of free maxicircle molecules which are preferentially labelled with [^{3}H]thymidine during a 20 min pulse. The specific radioactivity of these free maxicircle molecules is about 6 times higher than the network maxicircles. The free maxicircles represent roughly 10% of the total maxicircles in a mitochondrion. Restriction digests and fluorography of network and free maxicircles show striking differences in both the labelling of restriction fragments during [^{3}H]thymidine pulses and the stoichiometry of the restriction fragments. These results suggest that maxicircles replicate on the network by a rolling circle intermediate which produces free, linear molecules containing maxicircle sequences. Presumably these free linear molecules thread through the network and recircularize. [This research was supported by a postdoctoral fellowship from the Rockefeller Foundation to S.L.H. and NIH grant GM27608 to P.T.E.]

0077 THE REPLICATION OF KINETOPLAST DNA MINICIRCLES, Peter A. Kitchin and Paul T. Englund, Dept. of Physiological Chemistry, Johns Hopkins School of Medicine, Balto., MD 21205

Kinetoplast DNA, the mitochondrial DNA of trypanosomatids, consists of thousands of minicircles and a few maxicircles interlocked in a massive network. Previous work has indicated that minicircles are released from the network for the purpose of replication. We have confirmed and extended this observation by [^{3}H]thymidine pulse-chase studies of both network-bound and free minicircles. We have used agarose gel electrophoresis, in the presence of ethidium bromide, to isolate various forms of free minicircles for both biochemical and electron microscopic analysis. The free minicircles are resolved into nicked circles, covalently closed circles (of low superhelical density), linear double stranded molecules and a smear. All of these molecules contain minicircle sequences as shown by hybridization. During a pulse-chase experiment with [^{3}H]thymidine, the smear is labeled first (∿2 minutes), followed by nicked free minicircles (2-5 minutes). The label is then transferred to nicked minicircles bound to the network. The latter was determined by topoisomerase decatenation of networks. Later, during the chase, the covalently closed network minicircles become labeled and finally, after about 80-120 minutes, the label appears in the free covalently closed minicircles. Electron microscopic examinations of unfractionated free minicircles reveals the presence of many Cairns-type minicircle structures which probably give rise to the smear observed on agarose gels. We have not yet characterized the linearized minicircles. They may not be intermediates in the replication process but may have been produced by cleavage during the isolation process. [Supported by WHO grant 790184 and NIH grant GM27608-14.]

The Host-Parasite Pair

0078 MECHANISMS FOR RESISTANCE TO TRYPANOSOMA B. RHODESIENSE IN MICE. Antonie L.W. deGee and John M. Mansfield. Dept. Microbiol. and Immunol. School of Medicine, Univ. Louisville, Louisville, KY 40292

Animals are known to vary in susceptibility to trypanosomes depending on their genetic background. Inbred mouse strains differ in their antibody response (mainly IgM) to the surface antigen of T.b. rhodesiense LouTat 1.0, but it remains unclear the role such differences play in the observed variation in susceptibility. In order to elucidate the cellular basis of host resistance we made bone marrow chimeras between C3H/SW, C57Bl/10 and A-BY mice, all of which posses the H-2^b haplotype but differ in relative resistence to T.b. rhodesiense LouTat 1.0. The results are: (1) The IgM response of A-BY (intermediate) and C57Bl/10 (resistant) mice receiving C3H/SW (susceptible) bone marrow cells (C3H→A and C3H→C57) to variant antigen was lower than in all mice receiving C57Bl/10 bone marrow cells but all mice overcame the first parasitaemic wave. C3H→C3H mice, however, did not overcome the first peak and no IgM antibody was detectable. (2) C57Bl/10 bone marrow cells were capable of rescuing A-BY mice from early death but not C3H mice. Also, C3H→C57 mice survived significantly longer than C3H→A mice, which in turn survived longer than C3H→C3H mice. These results indicate the presence of 2 mechanisms of genetically determined resistence: one regulated by bone marrow derived cells and probably expressed through the humoral antibody response, and a second mechanism not related to bone marrow derived cells. In the most susceptable mouse strain (C3H background) both mechanisms are deficient while, in, for instance, A-BY mice only the bone marrow cell related mechanism fails.

0079 GENETIC CONTROL OF RESISTANCE TO MURINE MALARIA, M.M. Stevenson, S. Lemieux and E. Skamene, Montreal General Hospital Research Institute and Institut-Armand Frappier, Montreal, Quebec, Canada

Strain variation in the level of resistance to malaria was investigated in inbred mice after infection with Plasmodium chabaudi. Following intraperitoneal infection with 10^6-parasitized erythrocytes, mice of 11 inbred strains could be separated using survival time as the criterion into resistant (C57BL/6J, C57L/J, DBA/2J, CBA/J and B10.A/SgSn) and susceptible groups (A/J, DBA/1J, BALB/c, C3H/HeJ, AKR/J and SJL/J). Genetic analysis of F_1 hybrid and backcross progeny derived from one of the most resistant (B10.A) and from the most susceptible (A/J) strains as parents suggested that host resistance in this strain combination was genetically controlled by a single, dominant, non-H-2 linked gene. Inheritance of resistance to malaria was found to be autosomal but expression of the trait was influenced by the sex of the host, that is, female mice are more resistant than male mice. Analysis of the mechanisms of resistance to P. chabaudi showed: 1)phenotypic expression of the resistance gene was apparent within 6 days of infection as a significant difference between resistant and susceptible mice in the level of parasitemia; 2) the level of host NK cell activity was not related to the level of host resistance to malaria; 3) compared with susceptible A/J mice, resistant B10.A hosts had an augmented erythropoietic response during the course of malaria as well as during phenylhydrazine-induced anemia and 4) treatment with BCG or C. parvum resulted in an equal degree of protection, measured by parasitemia and survival, in both resistant and susceptible mice.

0080 MECHANISMS OF HOST RESISTANCE TO INFECTION WITH TRYPANOSOMA MUSCULI. Patricia Kongshavn, Fatima Vargas, Wally Rappatoni and Esfandiar Ghadirian. Department of Physiology, McGill University, Montreal, Quebec, Canada.

The course of infection with Trypanosoma musculi is characterized by a period of exponential growth (growth phase) followed by stabilization of the parasitaemia (plateau phase) and, finally, elimination of trypanosomes from the blood (elimination phase). Natural resistance to multiplication of the parasite during the growth phase has been investigated by a formal, genetic analysis of resistant and susceptible strains of mice, and shown to be under the control of a major gene which is not linked to the major histocompatibility complex. Acquired protective immune mechanisms have also been investigated and the crucial role of antibody has been established, using B-cell deficient mice. The mechanism of antibody-dependent elimination of T. musculi organisms has been shown to depend not on complement but, rather, upon the participation of a radiosensitive cell.

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0081 ISOLATION AND CHARACTERIZATION OF T-CELL CLONES SPECIFIC FOR *TRICHINELLA SPIRALIS* ANTIGENS. Christopher J. Krco, Ellen J. Abramson, Donald W. Wassom*, and Chella S. David, Dept. Immunology, Mayo Clinic, Rochester, MN 55905 and *Dept. Preventive Medicine, New York State School of Veterinary Medicine, Cornell University, Ithaca, NY 14850.

We have been conducting investigations with the ultimate goal of elucidating the genetic factors and cellular interactions necessary for an optimal *in vivo* response to *T. spiralis* infection. As an adjunct to *in vivo* studies we have characterized the basic prerequisites for optimal *in vitro* responses to solubilized *T. spiralis* antigens and have demonstrated a role for Ly-1+ T-cells and Ly-1,2,3+ amplifier cells as well as I-A and I-E immune response (Ir) gene molecules. We report the characterization of T-cell lines and clones specific for solubilized *T. spiralis* antigens from B10.K (H-2^k) mice. The proliferation responses of two clones (mm-A5 and mm-C1) are specifically inhibited by anti-I-E but not by anti-I-A monoclonal antibodies. The inhibition patterns are consistent with cis-gene complementation in clones mm-A5 and mm-C1 involving the A^k_e β-chain and the E^k α-chain of the I-E molecule. Inhibition is obtained with an anti-A^k_e specific monoclonal antibody (H9-14.8) but not with an A^k_α specific monoclonal antibody (10-2.16). Inhibition was also observed with anti-Ia.7 (H40-242) E^k_α specific and anti-Ia.22 (17-3-3) monoclonal antibodies. The inhibition patterns were confirmed by antigen presentation experiments using recombinant inbred mice. Only B10.K ($A^k_eE^k_\alpha$) and not B10.A(5R) ($A^b_eE^k_\alpha$) or B10.S(9R) ($A^s_eE^k_\alpha$) spleen cells could effectively present *T. spiralis* antigens to clones mm-A5 and mm-C1. These results demonstrate the role of "hybrid" or "combinatorial" Ia antigen determinants in conferring resistance to *T. spiralis* challenge.

0082 T LYMPHOCYTE CLONES SPECIFIC FOR LEISHMANIAL ANTIGENS Haynes W. Sheppard, Phillip A. Scott and Dennis M. Dwyer LPD, NIAID, NIH, Bethesda MD. 20205.

Studies in man and experimental animals suggests that cell mediated immunity is of primary importance in limiting the pathogenesis of cutaneous and visceral Leishmaniasis. In an attempt to determine, more directly, the role of T lymphocytes and the nature of the antigens which activate them, we have employed recently developed methods for the propagation of antigen specific T lymphocyte lines and clones. Preliminary results suggest that: (1) Murine T lymphocyte lines can be established which are specifically reactive with antigens present on intact Leishmania donovani promastigotes.(2) These lines cross-react extensively with promastigotes of other Leishmania species.(3) T lymphocyte clones can be isolated and about 40% are specific for L. donovani and very closely related species while about 60% are cross-reactive.(4) Some lines and clones passively transfer foot-pad DTH when injected locally and some secrete a lymphokine activity which elicits intracellular killing of amastigotes within infected macrophages.(5) The majority of the clones appear to recognize carbohydrate antigens and absorption with solid substrate-bound lectins indicates that the antigens contain mannose and galactose.(6) Although the majority of the clones are H-2 restricted, two clones appear to be reactive with allogeneic H-2 antigens as well as parasite antigens.(7) Two extensively cross-reactive monoclonal antibodies,(kindly provided by Diane McMahon Pratt and Charles Jaffe Harvard Medical School) which were raised after immunization with L. tropica and L. donovani respectively, can absorb the antigen to which several of the clones respond.

0083 NATURAL KILLER CELL ACTIVITY AGAINST INFECTIOUS FORMS OF TRYPANOSOMA CRUZI, Frank M. Hatcher and Jennifer D. Cobbs, Meharry Medical College, Nashville, TN 37208.

Previous investigations in our laboratory have demonstrated that epimastigote forms of Trypanosoma cruzi (extracellular dividing forms obtained from axenic culture) are susceptible to the cytotoxic activity of murine natural killer (NK) cells. We present herein, the results of experiments which suggest that infectious blood-form trypomastigotes (BFT) of T. cruzi are similarly susceptible to the cytotoxic activity of cells with the NK cell phenotype. Cytotoxicity assays were performed using cells from the spleens or peritoneal cavity of normal C57Bl/6 female mice, mice infected with 10^3 or 10^4 BFT, or mice stimulated with poly inosinic·cytidylic (poly I·C) acid (100 ug per mouse, 18-22 hours previously) cultured with 2×10^4 BFT obtained from stock infected mice. Cultures of parasites and cells or parasites in medium only (controls) were incubated 5-7 hours and subsequently examined for the number of viable parasites remaining. Cultures containing effector cells from poly I·C stimulated or infected mice had significantly increased cytotoxicity as measured by the reduction in parasite number and compared to that observed in assays containing cells from normal mice. Depletion of plastic adherent cells did not reduce anti-BFT cytotoxicity. Effector cell pretreatment with rabbit anti-mouse immunoglobulin and C' also had no effect on cytotoxicity, while cells pretreated with anti-Thy 1.2 plus C' had 10-30% less activity. Cells depleted of NK 1.2 surface antigen bearing subpopulations failed to demonstrate any cytotoxic activity against BFT of T. cruzi. These results indicate a potential role for NK cells in the control and elimination of this parasite during infection.

0084 EFFECT OF TRYPANOSOMA CONGOLENSE ON BOVINE SERUM COMPLEMENT AND BLOOD LEUKOCYTES, H. Tabel, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N OWO

Freshly isolated Trypanosoma congolense were mixed with fresh bovine serum and then frozen and thawed once. Bovine serum treated this way showed activation of complement via the alternative pathway by: (a) generation of $B_{\chi 2}$, a split product of factor B and (b) inhibition of lysis of guinea pig erythrocytes in the presence of 10 mM EGTA and 5 mM $MgCl_2$. Migration tests carried out with bovine peripheral blood leukocytes in RPMI 1640 media containing 30% fresh autologous serum treated with T. congolense as described above resulted in significant inhibition of leukocyte migration. No migration inhibition was observed when autologous serum had previously been heated at 56°C/10 min. It is suggested that migration of bovine leukocytes caused by disintegrated T. congolense organisms is mediated by a complement factor(s) generated by complement activation via the alternative pathway.

0085 RESISTANCE OF PATHOGENIC ENTAMOEBA HISTOLYTICA TO LYSIS BY HUMAN SERUM, Sharon L. Reed, Peter G. Sargeaunt and Abraham I. Braude, University of California Medical Center, San Diego, California 92103 and London School of Hygiene and Tropical Medicine, London, U.K. WC1 7HT

Although axenized strains of E. histolytica are killed by human serum, pathogenic E. histolytica can invade the blood stream, disseminate to the liver, and resist killing by complement in the blood and other body fluids. Now that E. histolytica can be separated into pathogenic and nonpathogenic strains by their zymodeme patterns, we made a comparison of susceptibility to lysis by human serum among five nonpathogenic and eleven pathogenic strains of E. histolytica . All strains of amoebae were maintainned in Robinson's medium where both trophozoites and cysts are produced. The trophozoites of the nonpathogenic strains were found to be uniformly susceptible to lysis with more than 70% killing in sixty minutes. No killing occurred in simultaneous samples with heat-inactivated serum. Nine of eleven pathogenic strains, including five strains isolated from liver abscesses, were found to be resistant to lysis by serum under identical conditions. The difference between pathogenic and nonpathogenic strains was significant at the 0.001 level (Student's t test). Resistance to complement-mediated lysis may be an inherent property of most pathogenic strains and may prove to be a necessary virulence factor for dissemination.

0086 SERUM CONTAINING TUMOR NECROSIS FACTOR (TNF) IS CYTOTOXIC FOR THE HUMAN MALARIA PARASITE, PLASMODIUM FALCIPARUM, C.G.Haidaris, J.D. Haynes, M.S. Meltzer, and A.C. Allison*, Dept. of Immunology, Walter Reed Army Institute of Research, Washington, DC 20012, and *Syntex Research, Palo Alto, CA 94303

Sera containing TNF, a soluble product of macrophage origin, were obtained by a standard procedure in which mice infected with Mycobacterium bovis (BCG) were bled 2 hours after intravenous administration of bacterial endotoxin (LPS). Varying concentraions of sera were added to triplicate 100 ul cultures of P. falciparum, and viability was assessed by measuring 3-H-hypoxanthine incorporation during the last 8 hours of the 96 hour culture period. At concentrations of 1 to 3% different preparations of TNF sera caused a 2 to 3-fold increase in hypoxanthine incorporation. At higher concentrations (4 to 6%) there was a precipitous drop in incorporation to 2 to 5% of control. Concurrent assays with control sera (normal, BCG, or LPS alone) caused some stimulation, but no inhibition at up to 6% concentration. Examination of cultures containing TNF serum after 72 hours of culture showed morphologic deterioration of parasites within erythrocytes. The presence of TNF was confirmed using a standard L-cell cytotoxicity assay. Others have shown that rodent malaria infections can enhance the ability of mice to produce TNF and to produce a non-antibody mediator of parasite killing. Our data suggest that similar factor(s) may play a role in immunity to human malaria.

0087 INHIBITION OF MITOGEN INDUCED LYMPHOCYTE BLAST TRANSFORMATION AND CELL PROLIFERATION BY EXCRETORY-SECRETORY PRODUCTS OF LARVAL ANISAKID NEMATODES, Richard B. Raybourne and Robert S. Desowitz, University of Hawaii, Honolulu, Hi. 96816

Excretory-secretory products were collected from supernatants of in vitro cultures of larval nematodes, Anisakis simplex type I and Terranova sp. Hawaii type A. Both of these larvae are histotropic, and produce gastric lesions if ingested by laboratory animals or man. These materials were partitioned according to molecular weight by membrane ultrafiltration. Extracts of intact larvae were also prepared. Both of these materials were tested in vitro for cytotoxic and cytostatic effects on cultures of concanavalin A and LPS stimulated rodent lymphocytes. Excretory-secretory materials were found to be more potent inhibitors of rat and mouse lymphocyte blast transformation, than whole larval extracts of the same parasites. Determination of cell viability and cell number before and after mitogen stimulation indicated that the inhibition of ^{3}H-thymidine incorporation observed above was due to cytostatic rather than cytotoxic effects on the proliferating lymphoid cells. The material(s) responsible for these effects are greater than 10,000mw, nondialyzable, and heat labile (80^{0}C one hour), suggesting a possible proteinacous nature. The significance of these observations may be that these materials, elaborated in vivo modulate the granulomatous response associated with the worm induced lesions.

0088 MALARIAL IMMUNE COMPLEXES INHIBIT Fc RECEPTOR-MEDIATED PHAGOCYTOSIS, Hannah Lustig Shear, New York University Medical Center, New York, N. Y. 10016.

Immune complexes are known to occur during malaria and are often found in the kidneys of infected hosts. We have partially purified immune complexes from the serum of mice heavily infected with Plasmodium berghei. The complexes sediment with the IgM peak on sucrose gradients and are excluded by Sephacryl S-300. The complexes contain several subclasses of mouse IgG and malarial antigen. We found that these complexes inhibit Fc receptor-mediated phagocytosis by normal macrophages, but not attachment to the Fc receptor or to the C3b receptor.

Splenic macrophages, isolated from P. berghei-infected mice appear to be coated with immune complexes. Like normal macrophages incubated with immune complexes, phagocytosis via the Fc receptor is inhibited in these splenic macrophages, whereas attachment of IgG-coated particles is enhanced and attachment of complement-coated particles is normal. However, when the splenic macrophages are incubated overnight, their ability to ingest via the Fc receptor is restored to higher than normal levels, suggesting that the immune complexes had been either ingested or eluted from their plasma membranes. These results suggest that immune complexes modulate the immune response to malaria by inhibiting immune phagocytosis and, perhaps, by interfering with other effector mechanisms.

0089 NON-IMMUNE KILLING OF GIARDIA LAMBLIA AND ENTAMOEBA HISTOLYTICA BY HUMAN MILK, Frances D. Gillin, David S. Reiner and Chi-Sun Wang, University of California at San Diego and Oklahoma Medical Research Foundation.

The clinical courses of both giardiasis and amebiasis are variable and serum antibodies do not appear to be protective. We propose that natural or immune factors either produced by intestinal tissue, transported into the intestine, or ingested (i.e., by breast fed babies) might promote resistance to these diseases. These factors may prove important in the small intestine where these parasites excyst prior to colonization. Human milk is very rich in secretory IgA (S-IgA) antibodies, as well as non-specific antibacterial factors (e.g., lactoferrin, lysozyme).

Both G. lamblia and E. histolytica trophozoites were killed by non-immune human milk (NHM) in a time and concentration-dependent manner. 100% of Giardia were killed in 3-4 hours with 0.3% milk or in less than 1/2 hour with 3% milk. Milk from five normal women was active, but fresh cow's or goat's milk did not affect the parasites (at 0.2 to 50%). Removal of >99% of the S-IgA from NHM did not decrease its Giardia-cidal activity. Thus, the killing was not antibody-dependent. Thermal inactivation experiments revealed both heat-labile and heat-stable factors in NHM. Studies of purified milk proteins indicate that the heat-labile factor may be an unusual lipase present in the milk of humans, but not of lower mammals. This is the first demonstration of non-immune antiparasitic defenses in human milk.

0090 PLASMODIUM FALCIPARUM MALARIA : ACTION OF THE SPLEEN AND OF ANTIBODY ON THE CYTOADHERENCE OF INFECTED ERYTHROCYTES, Peter H.David, Marcel Hommel, Iroka J.Udeinya and Louis H.Miller, Parasite Immunology Division, Harvard Medical School, Boston Ma 02130

In human malaria, the tissue sequestration of infected erythrocytes containing the more mature stages of the parasite (trophozoites and schizonts) is characteristic of Plasmodium falciparum infections. We have demonstrated a marked difference in the in vitro and in vivo cytoadherence properties of infected erythrocytes from intact and from splenectomized squirrel-monkeys infected with the same strain of P. falciparum.

In vitro, when infected blood is incubated with cultures of human melanoma cells, infected erythrocytes from intact animals and not from splenectomized animals bind to the melanoma cells. This correlates with a marked decrease of the in vivo sequestration of trophozoite- and schizont-infected erythrocytes in splenectomized animals.

In vitro binding can be reversed by immune monkey serum ; in parallel, the inoculation of infected intact animals with immune serum is followed by the appearance of trophozoite- and schizont-infected erythrocytes in the blood stream where normally only erythrocytes infected with early stages can be found.

These results indicate that the spleen modulates the alterations of the infected erythrocyte membrane responsible for parasite sequestration and suggest that the prevention and reversal of sequestration could be one of the mechanisms of antibody protection in malaria.

0091 ANTIGENIC VARIATION AND EXPRESSION OF VARIANT ANTIGEN WITH CLONED PLASMODIUM KNOWLESI REQUIRE THE HOST SPLEEN, John W. Barnwell, Russell J. Howard and Louis H. Miller,LPD, NIAID, NIH, Bethesda, Md., 20205. Variant antigens appear on Plasmodium knowlesi infected erythrocytes as the asexual parasite matures and are detected by antibody-mediated schizont-infected cell agglutination (SICA). We previously described a new phenotype of P. knowlesi in which uncloned parasites passaged in splenectomized (spx) monkeys were no longer agglutinable by immune sera [1]. This phenotype is designated SICA[-], and that expressing variant antigen SICA[+]. We have now shown that cloned SICA[+] parasites can switch to SICA[-] in spx-monkeys. This switch is slow, requiring several passages in spx-monkeys. After passage in one animal the agglutination titer decreased 4-16 fold. The decrease was associated with decreased antibody binding to all infected erythrocytes. Furthermore, we showed that cloned SICA[+] parasites can undergo antigenic variation in nonsplenectomized monkeys, but not spx-monkeys, in response to variant-specific antibody. The asexual malaria parasite can therefore alter expression of variant antigen in response to the host environment (anti-variant antibody or splenectomy).

Two courses of parasitemia were observed when cloned SICA[-] parasites were inoculated into intact animals: fulminant parasitemia (>20%) associated with conversion of the parasite from SICA[-] to SICA[+], or, control of parasitemia associated with retention of SICA[-] phenotype. We propose that the variant antigen functions to depress a spleen-dependent immune response. [1] J.W. Barnwell, R.J. Howard & L.H. Miller, 1982, J. Immunol. 128, 224-226.

0092 MONOCLONAL ANTIBODIES AGAINST VARIABLE ANTIGENS OF TRYPANOSOMA BRUCEI : CHARACTERIZATION AND USE, Peter Myler, Donna Birdsell, Nina Agabian* and Kenneth Stuart, Issaquah Health Research Institute, Issaquah, WA, 98027, and*University of Washington, Seattle, WA, 98195.

Monoclonal antibodies (McAbs) were produced by immunization with purified variant surface glycoproteins (VSGs) from 6 clones expressing different variant antigen types (VATs) of the ISTat 1 serodeme of T.brucei, and identified by reaction with the immunogen using a solid-phase ELISA. Several McAbs reacted specifically with each of the 6 VATs used, as determined from ELISA and indirect immunofluorescence assays (IFAs) against fixed and living trypanosomes. All specific McAbs were IgG and one cross-reacted with more than one VAT. The specificity of the McAbs was further examined primarily by "Western"blots.

VAT-specific McAbs were used to study the stability of the 6 VATs described during growth in lethally-irradiated mice. Three VATs (1.1, 1.7, and 1.A) were stable for at least 30 days during more than 10 passages through irradiated mice. Three other VATs (1.2, 1.5, and 1.11) underwent antigenic variation during this time. All three VATs appeared to express 1.A VSG after this switch and the times of appearance of 1.A and disappearance of the original VAT were characteristic to each VAT.

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0093 REPLICATION OF INFECTIOUS T. RHODESIENSE IN SUBCUTANEOUS CHAMBERS. Gonzalo Ballon-Landa and Charles E. Davis, Univ. of California, San Diego, San Diego, CA 92103

In an attempt to study the role of trypanosomal products in the pathogenesis of trypanosomiasis, we have developed an in vivo model that allows soluble substances to enter the circulation, while sequestering the whole organisms. Millipore chambers (18 mm diameter, 2 mm width, .45 u pore size) containing 10^4 trypanosomes/ml (T.b. rhodesiense, strain 853, ILRAD) in Eagle's medium were implanted subcutaneously in CF1 mice. When chambers were removed, motile trypanosomes were counted, sera collected for ELISA against the whole organism, and infectivity tested by injection into healthy mice.
The results of three experiments are given below.

	day 0	day 4	day 7	day 14
Mean number of motile trypanosomes/ml	4.4×10^4	4.3×10^6	3.9×10^6	1.4×10^6
Number of positive chambers/total		4/4	9/10	7/10

Motile trypanosomes harvested from chambers were always infectious. By day 21 IgG antibody titers of mice with infected chambers were equal to those of systemically infected mice at day 10.

We conclude 1) contact between trypanosomes and host cells is not essential for survival and multiplication of infectious trypanosomes in vivo, and 2) mice develop antibody to soluble trypanosomal products that diffuse across 0.45 u membranes. This system should facilitate studies of the roles of "toxins" in the pathogenesis and immunology of African trypanosomiasis.

0094 SPOROZOITES RECOGNIZE SPECIFIC HEPATOCYTE RECEPTORS, Michael R. Hollingdale, Biomedical Research Institute, Rockville, MD 20852 and Alan L. Schwartz, Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

Specificity of malarial parasites for their target cell is probably determined by interaction of parasite surface ligands with plasma membrane receptors. Receptor mediated endocytosis defines a sequence of events for the movement of macromolecules, including parasites, to intracellular environments. Initiation of infection with malaria begins with the injection of sporozoites, and their invasion of liver parenchmal cells. Sporozoites are uniformly coated with circumsporozoite (CS) proteins, which contain species-specific epitopes, but share considerable peptide homology. Monoclonal antibodies, and monovalent fab fragments, against CS proteins abolish sporozoite infectivity *in vivo* and *in vitro*, suggesting that CS proteins play a role in parasite infectivity. Recently, a cloned human hepatoma cell line (HepG2-A16) has been shown to have surface receptor properties that are indistinguishable from primary hepatocytes. Sporozoites infect HepG2-A16 cells, and monoclonal and Fab fragments to CS proteins block both attachment and entry of rodent, simian and human malarial sporozoites. Thus, immunoprophylaxis of malaria based on a sporozoite vaccine appears to depend, at least in part, upon antibody inhibition of CS protein recognition of a hepatocyte receptor. Receptor function has significance and finds expression in terms of the dynamic interactions between receptor molecules, their ligands (CS proteins) and other membrane constituents. Characterization and isolation of the hepatocyte receptor for sporozoites will lead to a better understanding of the mechanism of malaria infection, and its interruption by specific immune responses.

This work was supported by AID Contract DSPE-C-0079.

0095 OVALOCYTES : AN ERYTHROCYTE VARIANT NATURALLY SELECTED FOR RESISTANCE TO ALL HUMAN MALARIAS. Gretel Lamont, Allan Saul and Chev Kidson. Queensland Institute of Medical Research, Brisbane, Australia, 4006.

The response of the human host in evolutionary time to malaria has led to selection of a range of erythrocyte mutants. Of these, Melanesian ovalocytes are possibly the most intriguing, by virtue of their evident resistance to four major malaria parasite species : P. falciparum, P. vivax, P. malariae, P. knowlesi. Ovalocytic individuals consitutte up to 20 percent of some population groups in areas of Papua New Guinea holoendemic for malaria. These mutants are genetically dominant and involve alterations in the erythrocyte membrane. They are unusually stable to heat, do not crenate, are resistant to drug-induced endocytosis but membrane fluidity and cellular deformability are normal. Thus, some properties consistent with a cytoskeletal abnormality, could be pertinent to their resistance to parasite invasion, e.g. the reduced endocytosis. However, similarly reduced endocytosis in spherocytes is not associated with resistance to malaria. Other evidence points to the presence of surface alterations in ovalocytes. Some blood group antigens are selectively depressed, including En(a), Wr(b), S-s-U which have been suggested by some investigators to be involved in erythrocyte receptors for merozoites. However, purified ovalocyte and normocyte glycophorins inhibit parasite invasion equally well. Competition experiments have shown that P. falciparum merozoites bind very poorly to ovalocytes, consistent with alteration of surface receptor(s). Since the major malaria parasite species have different receptor requirements and ovalocytes exhibit broad spectrum resistance, the mutation must affect properties common to several receptors. It is proposed that altered receptor accessibility is the basis of ovalocyte resistance to malaria.

0096 PLASMODIUM FALCIPARUM INVASION OF ERYTHROCYTES
Margaret E. Perkins, Rockefeller University, New York, N.Y. 10021.

Purified Glycophorin A and Glycophorin B inhibit invasion of P.falciparum merozoites into human erythrocytes(J.Cell Biol.90,563-567). Desialylated Glycophorin retains partial activity in blocking invasion. Thus it is proposed that the merozoites recognize both Glycophorin A and B during entry and that the specificity of this interaction resides partly in the sialic acids and partly in the remainder of the polypeptide. Using a fraction of Glycophorin as a probe to identify possible merozoite surface proteins involved in erythrocyte recognition, we have identified two proteins which specifically bind to the sialoglycoproteins. A 150kd protein is totally adsorbed to the Glycophorin and can only be removed fully by 0.5% SDS. In addition a 200kd protein is also fully adsorbed to the Glycophorin fraction but can be removed by 1M urea indicating that the binding affinity is not as strong. In control experiments, none of these proteins bound to another sialoglycoprotein, fetuin.

In studies to examine the mechanism of erythrocyte deformability during merozoite invasion, we find that reagents which modulate membrane protein mobility, have a marked effect on the invasion of P. falciparum merozoites.

0097 INTESTINAL EPITHELIUM: CHANGES IN BRUSH BORDER MEMBRANE SURFACE AND TRANSPORT PROPERTIES PARALLEL IMMUNE REJECTION OF INFECTIVE LARVAE OF TRICHINELLA SPIRALIS, Gilbert A. Castro, Graham Bullick and Yael Harari, University of Texas Medical School, Houston, TX 77030

Immunity to Trichinella spiralis in the rat involves noninjurious rejection of infective larvae within 15 min postchallenge. The hypothesis being tested is that an altered physiological state in host gut epithelium, controlled by immune elements, effects this "rapid rejection" (RR) response. It is assumed that such envolvement should be reflected in changes in epithelial structure and/or function. Such changes were experimentally demonstrated focusing on two cell membrane-related phenomena. Maximum specific binding (B_{max}) of ^{125}I-Wheat Germ Agglutinin by epithelial brush border membranes (BBM) was reduced to 40-50% of the pre-infection level in rats immunized to T. spiralis by primary (1^{o}) infection. Decreased B_{max} (a) associated with a reduction in BBM sialic acid available for lectin binding, (b) was evident 2 wk after the immunizing infection, and (c) was sustained for at least 90 days. In addition to this change, acute alterations in epithelial function were observed following a secondary (2^{o}) infection. Low challenge doses (50 larvae) administered to immunized hosts caused a rapid reduction (within min), from prechallenge level, of electrogenic, mucosa-to-serosa Na^{+}:ß-methyl-D-glucoside cotransport, as monitored using Ussing-type chambers. Thus, two kinds of changes in epithelium are concluded to be associated with the capacity to express the RR response: (a) a structural change at the apical membrane surface that is induced by 1^{o} infection and chronically maintained, and (b) a functional change elicited immediately upon 2^{o} contact with infective larvae (Supported by NIH Research Grant, AI-11361).

0098 MATERNAL INFLUENCE ON FILARIAL INFECTION (BRUGIA MALAYI) IN GERBILS. A. Faye Schrater, Smith College, Northampton, MA 01063, Andrew Spielman, and Willy F. Piessens, Harvard School of Public Health, Boston, MA, 02115.

We examined the consequences of parental infection by determining in progeny, the prevalence of microfilaremia subsequent to infection with third stage larvae. Female progeny of infected parents generally became microfilaremic whereas those of non-infected parents rarely did so. Male progeny developed microfilaremic infections regardless of parentage, although descendents of infected parents had higher parasitemias. We concluded that B. malayi infection in the mother predisposes the progeny to microfilaremia, possibly due to in utero exposure to filarial antigens resulting in immunological tolerance.

0099 "CLONING OF A CHROMOSOMAL VIRULENCE DETERMINANT OF HAEMOPHILUS INFLUENZAE TYPE b" E.Richard Moxon, Robert A. Deich, Carla Connelly. Johns Hopkins University, Baltimore, MD. 21205, Frederick Cancer Research Facility, Frederick, MD 21701.

H. influenzae (Hi) is the most common cause of bacterial meningitis in the United States and elsewhere. Serotype b strains, which elaborate a capsule composed of a linear polymer of polyribosyl-ribitol phosphate (PRP), account for more than 90% of these infections. We have constructed a library of chromosomal fragments of Hi in Charon 4 and have isolated two independent clones (designated 48 and 60). Using DNA transformation and a well characterized animal model (rat) we have demonstrated the role of chromosomal genes in the virulence of Hi. Clones 48 and 60 contain, respectively, 13.5 kilobase (kb) and 15 kb inserts of Hi DNA which contain sequences necessary for type b capsule elaboration. When an avirulent, capsule-deficient Hi strain (S2) was used as a recipient, donor DNA from either clone 48 or 60 resulted in transformation to serotype b organisms. These type b transformants were highly virulent and caused bacteremia and meningitis in rats following intranasal inoculation, (untransformed S2 were avirulent). The nucleotide sequences responsible for type b transformation reside upon a 4 kb Eco RI fragment of clone 48 or 60. Southern hybridization, using the 4 kb fragment as a probe, showed that genomic DNA from Hi type b strains had unique Eco RI fragments homologous to the probe, whereas capsule-deficient mutants had no homology. Thus, we conclude that one or more genes coding for type b capsule elaboration and virulence of Hi have been cloned. Following intranasal inoculation of rats with appropriately transformed or untransformed strains, we have clearly shown that the cloned DNA possesses sequences which are biologically relevant to nasopharyngeal colonization, intravascular survival, and CNS invasiveness of H. influenzae.

0100 GENETIC ANALYSIS OF OUTER MEMBRANE ANTIGENS OF SALMONELLA TYPHIMURIUM AND THEIR ROLE IN HOST PROTECTION, Karen A. Shalala and Richard L. Warren, Wright State University, Dayton, Ohio 45435

Protection against lethal salmonellosis in mice is associated with the induction of cell-mediated immune response. Introducing deep rough mutations into avirulent strains of Salmonella typhimurium has resulted in the loss of protective capacity of those strains. Since the deep rough mutations result in defective LPS synthesis and an overall reduction in outer membrane protein (OMP) content, it has been difficult to assign the loss in protective ability of the strains with a specific alteration in the outer membrane. In order to assess the role of specific outer membrane components in host protection, OMP mutants of S. typhimurium vaccine strain (RIA) have been constructed by P22 mediated transposition of Tn10. Since the RIA strain is a smooth variant, direct selection of OMP mutants by phage resistance was not possible. Alternatively, mutants in certain outer membrane proteins have altered sensitivities to antibacterial agents which use the proteins as diffusion pores. The following agents were used to screen all Tn10 insertion mutants: cephaloridine, chloramphenicol, crystal violet and tetracycline. Seven classes of mutants were obtained based on different phenotypes. The cosmid vector DPT55 was used to clone the OMP mutations into E. coli for further analysis. Clones were identified by hybridization and their ability to express the mutant phenotype. The specific products of the OMP mutants are being characterized by expression of the cloned fragments in E. coli maxicells. By construction and characterization of single site mutations in various outer membrane components of a vaccine strain, the contribution of any one of these in eliciting protective immune response can be more clearly defined.

0101 STRATEGIES FOR IDENTIFYING VIRULENCE FACTORS OF TRICHOMONAS VAGINALIS. John F. Alderete, Ph.D.; Univ. Texas Hlth. Sci. Ctr.; San Antonio, Texas. The study of the regulation of expression of specific Trichomonas vaginalis virulence determinants with defined biologic function - such as proteins responsible for either parasite-mediated host tissue adherence or cell cytopathogenicity - is a goal of our research program. Thus, our initial strategy was directed toward characterization of parasite proteins and identification of highly immunogenic trichomonal membrane components. Analysis of several human strains of Trichomonas vaginalis was accomplished using electrophoretic techniques. Complex profiles were obtained for all strains examined with proteins ranging in MW from 200,000G (200K) to 20K. Highly sensitive and specific radioimmunoprecipitation procedures were then employed using intrisically versus extrinsically-labeled whole organisms, detergent extracts, or water-soluble protein preparations as a source of antigen. Immune rabbit sera or pooled sera from subcutaneously-infected mice contained high-titered antibody which reacted with numerous high and low MW proteins. Additionally, individual challenged mice were found to possess identical antibody responses to these immunogenic trichomonal proteins. A high degree of serologic crossreactivity among the various strains was observed. Antigenic homology was also detected between pathogenic human T. vaginalis, the nonpathogenic T. tenax, and the virulent bovine trichomonad, T. foetus KV-1. This study represents one of several approaches by our laboratory aimed at defining factors responsible for trichomonal disease pathogenesis. These initial data are prerequisite for future examination of the genetic regulation of virulence determinants.

0102 TRANSPOSON MUTAGENESIS OF PSEUDOMONAS AERUGINOSA, Marilyn J. Stapleton and Richard L. Warren, Wright State University, Dayton, Ohio 45435

Extracellular enzymes have been implicated as possible virulence factors in Pseudomonas aeruginosa. Any one of several compounds including alkaline protease, elastase and exotoxin A contribute to the disease process. Since the production and secretion of exoenzymes represents genetic regulation and since virulence is multi-faceted, the dissection of P. aeruginosa virulence factors and their controlling genetic elements is essential in determining the contribution of any one virulence factor to pathogenicity. In order to define the host-parasite interaction, mutants deficient in a specific toxic or proteolytic activity are necessary. A "suicide plasmid" mutagenesis procedure was used to generate protease-deficient mutants in P. aeruginosa. The association of the mutant phenotype with Tn 5 insertion events was demonstrated by cotransduction into a wild type P. aeruginosa strain. Several independent Tn 5 insertion mutants had reduced protease production, partially impaired elastase activity and no immunologically reactive alkaline protease. Once the Tn 5 was inserted into the P. aeruginosa genome, the frequency of its transposition to a new site was 10^{-8}. Kanamycin resistance was stable in approximately 50% of the exconjugants. Spontaneous kanamycin resistance occurred about one-tenth as frequently as Tn 5-induced mutants; however, none of these were protease-deficient. Hybridization of a Tn 5-radiolabelled probe to genomic DNA digests from putative insertion mutants provided physical evidence for the presence of Tn 5. These mutants will be assayed for virulence in an animal model system.

0103 THE SIGNIFICANCE OF COLONIAL MORPHOLOGY PHENOTYPES ON THE VIRULENCE OF THE CELL WALL-LESS PROCARYOTE, MYCOPLASMA PULMONIS. Alan Liss, Department of Biological Sciences, SUNY at Binghamton, Binghamton, N.Y., 13901. Mycoplasmas are a diverse group of procaryotes sharing the property of the absence of a cell wall. Being bound solely by a single biological membrane, mycoplasmas represent a model for host-parasite interactions with bacterial-like and eucaryotic-like parameters. Recently I have observed stable phenotypic variation in the colonial opacity of Mycoplasma pulmonis, the etiologic agent of murine respiratory mycoplasmosis. The limits of opacity were designated opaque (op) and transparent (tr). Each phenotype can be generated from the other. Colonial opacity was found linked with several biological characteristics including sensitivity to infection by the M. pulmonis virus. Whole cell polypeptide analyses of pure culture op- and tr-variants revealed differences. Endonuclease digestion of tr-variant DNA showed specific differences when compared to op-variant DNA treated and banded using similar procedures. Perhaps of in vivo significance is the observation that tr-variants predominate in fresh clinical M. pulmonis isolates (5 of 5 isolates investigated) while op-variants predominate in high passage strains (3 of 3). Further work is being done to characterize these mycoplasmas. The significance of these observed phenotypes with M. pulmonis pathogenicity is also being investigated, using several models, including virulence in chick embryos and cytotoxity in a tissue culture system.

0104 COORDINATION OF PROTEIN SYNTHESIS IN AN OBLIGATE INTRACELLULAR PROKARYOTIC PARASITE, R.L. Zuerner and H.A. Thompson, West Virginia University, Morgantown, WV 26506. Studies on cell-free extracts from Coxiella burnetii suggest that the extracellular organism contains no messenger RNAs but does contain an active translational apparatus. When inside eukaryotic host cells, the organisms grow within phagolysosomes where the acid pH is a requisite for their metabolism; this environment can be approximated in vitro (Hackstadt and Williams, PNAS 78:3240-3244, 1981). A rapid lysis method has been devised to obtain purified C. burnetii grown in Baby Hamster Kidney (BHK-21) cells, which allows recovery of parasites of greater stability such that they more nearly approximate intracellular conditions. When parasites are rapidly isolated in this manner and incubated with a carbon source plus amino acids at low pH, transcription and polypeptide synthesis is initiated and continues for 4-6 hr. Fifteen to 20 polypeptide species are synthesized, as evidenced by the concentration-dependent incorporation of ^{3}H leucine or ^{35}S methionine into these species. Coxiella burnetii obtained from naturally lysed cells (found in the medium) can likewise be induced to synthesize macromolecules by activation in acid. However, these previously released parasites synthesize macromolecules for 24 hr periods and the polypeptide patterns differ, as do their sensitivities to nucleic acid and protein synthesis inhibitors. In either case, two polypeptides are secreted from the cell cotranslationally during the activation process. The collective results suggest that media-derived and host cell lysate-derived C. burnetii represent two different phases of the life cycle of the parasite, and that biosynthesis of proteins under acid conditions in vitro is representative of early events after phagosome-lysosome fusion in natural infections.