ROUND-TABLES

[November, 2005-11-08 - 10h30 - ROOM A]

RT01A - Developmental regulation of endocytosis in trypanosomes

Mark C Field *(University of Cambridge)*

In Trypanosoma brucei, the African trypanosome, endocytosis is developmentally regulated and is substantially more active in the mammalian infective stage compared with the insect vector form. In the former stage endocytosis is likely part of the immune evasion system. The best characterized membrane recycling routes in trypanosomes are mediated by the small GTPases Rab4 and Rab11, but the precise roles that these pathways play have not been fully elucidated. Here we describe the use of RNA interference to assess the roles of these proteins in recycling in trypanosomes. For Rab11, we find that in the mammalian stage silencing had no effect on exocytosis of newly synthesized VSG, fluid-phase endocytosis, or transferrin uptake, but export of internalized transferrin was inhibited. By contrast, in insect stages, depletion of TbRAB11 blocked both fluid-phase endocytosis and internalization of surface proteins. For Rab4 we found that in the mammalian stage the GTPase was primarily involved in regulation of fluid-phase traffic to the lysosome but not in receptor-mediated endocytosis or recycling, distinct from the major roles in higher eukaryotes. By contrast, in insect stages Rab4 appears to mediate important recycling pathways. These data both demonstrate developmental regulation of recycling systems in trypanosomes and highlight evolutionary differences in function between sequence orthologues. Implications of these findings for immune evasion and virulence mechanisms will be discussed.

[November, 2005-11-08 - 10h30 - ROOM A]

RT01B - Endocytic portals in *Trypanosoma cruzi*

M. J. Soares *(Fundação Oswaldo Cruz)*

Endocytosis in trypanosomatid protozoa occurs through the flagellar pocket. In *Trypanosoma cruzi*, clathrin coated and uncoated vesicles arise from this region (JR Correa, unpublished data; Soares et al., 1992). However, an alternative endocytic portal occurs in *T. cruzi* epimastigotes and amastigotes: a cytostome/cytopharynx. The cytostome is a round opening at the anterior end of the parasites, from which a set of subpellicular microtubules runs inwards the cells, forming a long tube (the cytopharynx) that extends to the nuclear region, occasionally surpassing the nucleus. Endocytic vesicles bud off from the bottom of this structure. A freeze-fracture image in De Souza (1980) demonstrates that the cytostome is located outside the pocket. High-resolution field emission scanning electron microscopy showed that Concanavalin-A binds to surface proteins, possibly endocytic receptors, which are enriched at the cytostome membrane (Nakamura et al., 2005). It has been demonstrated that receptor-mediated endocytosis of transferrin occurs preferentially through the cytostome (Porto-Carreiro et al., 2000), but it has been shown that transferrin-loaded vesicles arising from the bottom of the cytopharynx are uncoated (Figueiredo and Soares, 2000). Recent work from our lab allowed to detect flotillin, a protein typically found in lipid rafts, in lipid-insoluble membrane fractions of *T. cruzi* epimastigotes (JR Correa, G Atella and MJ Soares, unpublished data). We suggest that receptor-mediated endocytosis of transferrin through the cytostome/cytopharynx occurs via caveolae/lipid rafts and that these vesicles fuse directly to the reservosomes. Indeed, the long cytopharynx allows budding of vesicles close to the reservosomes. It is noteworthy that a pioneer freeze-fracture work (De Souza et al., 1978) showed that the membrane of the reservosomes presents very few intramembranous particles. The finding of lipid raft domains in reservosome membrane fractions would support our hypothesis. Supported by CNPq and FIOCRUZ.

[November, 2005-11-08 - 10h30 - ROOM A]

RT01C - Molecular Determinants of Flagellar Membrane Protein Localization in Trypanosomes

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The eukaryotic flagellar membrane has a chemical composition different from that of other domains of the plasmalemma. Our work demonstrates that the specialized composition of the flagellar membrane reflects increased concentrations of lipid rafts, discrete regions of lateral heterogeneity in plasma membranes characterized by detergent resistance and high liquid order. In trypanosomes, a dually acylated calcium sensor which preferentially accumulates in the flagellum, shows the characteristics of a raft-associated protein. Other raft components, such as sterols and sphingolipids are also enriched in the flagellar membrane although GPI-anchored proteins are not. Direct visualization of membrane order by two-photon fluorescence microscopy, employing the amphiphilic order-sensing fluor laurdan, showed a markedly higher degree of liquid order in the flagellar membrane relative to other plasma membrane domains. The flagellar localization of dually-acylated flagellar membrane proteins could be altered by sterol depletion, or by chemical modulation of membrane fluidity. Detergent-extracted cells yielded discrete membrane patches localized to the surface of the flagellar axoneme, suggestive of intraflagellar transport particles. Together, these results provide biophysical and biochemical

evidence indicating that lipid rafts are highly enriched in the trypanosome flagellar membrane, providing a unique mechanism for flagellar protein localization and illustrating a novel means by which specialized cellular functions may be partitioned to discrete membrane domains. With respect to the localization of dually acylated flagellar calcium-binding proteins (FCaBP in T. cruzi and calfagin in T. brucei), the acylation state of the protein is critical for its localization. The apoprotein has a cytoplasmic localization, the myristoylated protein is located in the pellicular membrane and the myristoylated-palmitoylated protein is located in the flagellar membrane. Thus, a combination of protein acylation and the particular lipid environment of specific surface membrane domains appear to allow specific targeting of membrane proteins to their correct locations in the trypanosome cell.

[November, 2005-11-08 - 10h30 - ROOM A]

RT01D - Reservosomes: multipurpose organelles?

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Reservosomes are big compartments present at post-nuclear region of epimastigote forms of parasites belonging to the genus *Trypanosoma*, sub-genus *Schyzotrypanum.* Each epimastigote presents several reservosomes and, although its morphology can vary according to the growth conditions and parasite strain, usually it is a spherical organelle surrounded by a unit membrane. Reservosome matrix is slightly dense, mainly made of proteins, and presents electronlucent lipid inclusions (Soares and De Souza, J Submicrosc Cytol Pathol, 20:349, 1988). Reservosomes were first described as storing organelles because all macromolecules ingested by the parasite through an endocytic process, accumulate inside them (Soares and De Souza, Parasitol. Res. 77: 461, 1991) and are eventually degraded. Subsequently, reservosomes were found to be the main functional site of cruzipain in epimastigotes (Souto-Padrón et al, J. Cell Sci. 96: 485, 1990), pointing to a lysosome function, although no other lysosome marker has been identified in the organelle. Afterwards, in a very elegant work, Soares and co-workers (J. Cell Sci. 102: 157,1992) used the DAMP technique to evaluate as 6.0 reservosome pH at electron microscopy level, and compared reservosomes to mammalian late endosomes. Thirteen years after, no distinct lysosomes have been found in *T. cruzi* epimastigotes and reservosomes have been referred as lysosomes by several authors. We could also suggest a recycling ability of reservosomes by the localization of TcRab11 (Mendonça et al., Gene 243: 179, 2000), a small GTPase with high similarity with mammalian Rab11, a marker of recycling endosomes, and the absence of TcRab7 (Araripe et al., Biochem Biophys Res Commun. 321: 397, 2004), similar to Rab7, the GTPase characteristic of mammalian late endosomes. Recently, we have managed to demonstrate transferrin recycling from late endocytic compartments, possibly from reservosomes. All these data indicate that reservosomes need to be biochemically characterized, issue that we have already started (Cunha-e-Silva et al., FEMS Microbiol Lett. 214: 7, 2002). The first biochemical analyses have shown that reservosomes accumulate lipids, besides proteins. Reservosome electronlucent inclusions may assume a crystalloid format, suggesting that the large amount of lipids in the organelle may lead to heterogeneous arrangements, creating differentiated lipid domains that may have a functional role. In this way, protocols for lipid rafts identification and isolation was applied to purified reservosomes, yielding a detergent resistant fraction enriched in cholesterol and sphingolipids. Starting from total reservosome purified fraction, we also prepared a reservosome membrane fraction, aiming to distinguish its molecules from that belonging to the soluble content, thus making it easier the proteomic analyses and the establishment of a marker molecule. During metacyclogenesis, when epimastigotes differentiate to infective trypomastigote forms, reservosome content is massively degraded and the organelle disappears. Thus, it is very interesting to investigate what happens with the organelle during differentiation to trypomastigotes and how it is formed in the reversed process, when epimastigotes originate from trypomastigotes in the insect digestive tract. Both issues have already been addressed in our lab (Soares et al., Parasitol Res. 75: 522, 1989, Sant'Anna et al, Microsc Microanal. 10:637, 2004). We have managed to establish a reproducible protocol to the metacyclogenesis reverse, obtaining epimastigotes directly from metacyclic trypomastigotes. In this process, new reservosomes seem to originate from Golgi complex at the parasite's anterior region and then migrate to the posterior region. New reservosomes are already acidic and present cruzipain. Associating biochemical, morphological and molecular biology techniques we are now investigating how cruzipain is addressed to new reservosomes. It seems likely that reservosomes in an epimastigote do not constitute a uniform organelle population, with storing, recycling and lysosome typical functions varying with time and space.

[November, 2005-11-08 - 10h30 - ROOM B]

RT02A - *N*-Oxide containing heterocycles as trypanocidal drugs

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Chagas' disease or American trypanosomiasis is a serious public health problem in the countries and areas where it is endemic (21 countries in Central and South America) because there are no effective immunoprophylaxis or chemotherapeutic methods. Currently, clinical pharmacology of Chagas' disease is limited primarily to two drugs: the free radical producers Nifurtimox (Nfx) and Benznidazole. The therapy with these agents is considerably limited, including variable efficacy-restricted to the acute phase with

uncertain results during the chronic phase-, toxicity, resistance and supply problems. Our initial goal in the anti-trypanosomal agents research was to explore the bioactivities of *N*-oxide containing heterocycle derivative to find out new substances with less side effects than Nfx [1,2]. Physicochemical properties, such as lipophilicity and electrochemical behavior, were studied in order to explain the biological activity. We have also carried out three dimensional quantitative structure-activity relationship (3D QSAR) studies on *in vitro* antiparasitic activities against *Trypanosoma cruzi* to establish the pharmacophore for one of the family developed [3]. From the SAR and QSAR analysis we have designed and developed new generation of *N*-oxide containing heterocycle derivatives with high potent anti-trypanosomal activity and less toxicity [4,5]. Currently, clinical development of arylethenylbenzofuroxan derivatives as drugs for Chagas disease has been performed. In these sense, the following studies have been developed: 1) *in vivo* activity against a range of *T. cruzi.* 2) *in vitro* metabolic studies. 3) *in vitro* toxicity studies tests, Ames test, *in vitro* micronucleus and chromosome aberration test. [1] Cerecetto, H.; Di Maio, R.; González, M.; Risso, M.; Saenz, P.; Seoane, G.; Denicola, A.; Peluffo, G.; Quijano, C.; Olea-Azar, C. 1,2,5-Oxadiazole *N*-oxide Derivatives and Related Compounds as Potential Antitrypanosomal Drugs. Structure-Activity Relationships. J. Med. Chem. 1999, 42, 1941-1945. [2] Aguirre, G.; Cerecetto, H.; Di Maio, R.; González, M.; Porcal, W.; Seoane, G.; Denicola, A.; Ortega, M. A.; Aldana, I.; Monge, A. Benzo[1,2-c]1,2,5-Oxadiazole *N*-oxide Derivatives as Potential Antitrypanosomal Drugs. Structure-Activity Relationships. Part II. Arch. Pharm. 2002, 335, 15-21. [3] G. Aguirre, L. Boiani, M. Boiani, H. Cerecetto, R. Di Maio, M. González, W. Porcal, A. Denicola, O. E. Piro, E. E. Castellano, C. M. R. Sant'Anna, E. J. Barreiro. New Potent 5-Substituted Benzofuroxans as Inhibitors of *Trypanosoma cruzi* Growth. Quantitative Structure-Activity Relationship Studies, Bioorg. Med. Chem., 2005, in press. [4] G. Aguirre, L. Boiani, H. Cerecetto, R. Di Maio, M. González, W. Porcal, L. Thomson, V. Tórtora, A. Denicola, M. Möller. Benzo[1,2-c]1,2,5-Oxadiazole *N*-oxide Derivatives as Potential Antitrypanosomal Drugs. Part III. Substituents-Clustering Methodology in the Search of New Active Compounds, Bioorg. Med. Chem., 2005, in press. [5] G. Aguirre, H. Cerecetto, R. Di Maio, M. González, M.E. Montoya Alfaro, A. Jaso, B. Zarranz, M.A.l Ortega, I. Aldana, A. Monge-Vega. Quinoxaline *N,N'*-Dioxide Derivatives and Related Compounds as Growth Inhibitors of *Trypanosoma cruzi.* Structure-Activity Relationships, Bioorg. Med. Chem. Lett., 2004, 14(14), 3835-3839. *Drugs for Neglected Diseases Initiative Project*: Clinical Development of Arylethenylbenzofuroxan Derivatives as Drugs for Chagas Disease Dpto. Quimica Orgânica, Facultad de Quimica-Facultad de Ciencias, Iguá 4225, 11400 Montevideo, Uruguay. hcerecetfq.edu.uy, megonzalfq.edu.uy

[November, 2005-11-08 - 10h30 - ROOM B]

RT02B - Isoprenoid and sterol biosynthesis inhibitors as specific chemotherapeutic agents for Chagas disease: an update

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Among the most advanced agents in development for the specific treatment of Chagas disease are sterol and isoprenoid biosynthesis inhibitors, as this parasite has an essential requirement for specific endogenous sterols (ergosterol and its 24-ethyl analog) for survival and cannot use the abundant supply of cholesterol available in its mammalian hosts. We have shown that new triazole derivatives, which are potent and selective inhibitors of fungal and protozoan cytochrome P-450-dependent C14α sterol demethylase, are capable of inducing radical parasitological cure in murine models of acute and chronic Chagas disease, the first compounds ever to display such activity. Posaconazole (Schering-Plough), a structural analogue of itraconazole that is currently in Phase III clinical trials as a systemic antifungal, is a prime candidate for clinical trials in Chagas disease patients. TAK-187 (Takeda Chemical Co.) is a long lasting triazole derivative with broad-spectrum antifungal activity, which has very potent anti-*T. cruzi* activity in vitro and is capable of curing both acute and chronic infections in murine hosts even when the infecting strain is nitrofuran- and nitro-imidazole resistant; more recent work has shown than this compound is superior to benznidazole in preventing cardiac damage in a murine model Chagas disease. Albaconazole (Uriach y Compañia) is another potent fungal and protozoan C14α sterol demethylase inhibitor with remarkable in vitro anti-*T. cruzi* activity ; although its very short half life in the mouse ($<$0.5 h) precluded in vivo studies in this animal model, recent work in a dog model have demonstrated that the compound has curative activity in established infections of the virulent Y strain of *T. cruzi* with very low toxicity, although drug resistance was encountered with the Berenice-78 strain. Finally, ravuconazole (Eisai) has also been shown to be very active against *T. cruzi* in vitro, but its in vivo activity in mice was limited, probably due to inadequate pharmacokinetic properties in this animal model; however, these results do not necessarily rule out the potential utility of this compound in the treatment of human *T. cruzi* infections, as its minimal inhibitory concentration against intracellular amastigotes (1 nM) is $>$1,000-lower than the levels attainable in human plasma with multiple oral dosing and its terminal half-life in man is $\geq$120 hrs. Amiodarone is the most frequently used anti-arrhythmic compound in chronic Chagas disease patients. It has recently been shown that this compound also has intrinsic antifungal activity and potentiates the antiproliferative activity of ergosterol biosynthesis inhibitors. Work in our laboratories found that amiodarone inhibited the proliferation of both *T. cruzi* epimastigotes and intracellular amastigotes cultured *in vitro*, and acted synergistically with posaconazole against the amastigotes. Further work showed that amiodarone reduced the parasitemia and increased survival of acutely infected mice, indicating trypanocidal activity in vivo. Amiodarone disrupted the parasites' Ca^{2+} homeostasis but also blocked ergosterol biosynthesis, while posaconazole also affected Ca^{2+} homeostasis.

These results provide logical explanations for the synergistic activity of amiodarone with azoles against *T. cruzi* and suggested that anti-arrhythmia treatment of Chagas disease patients with amiodarone could have the added benefit of reducing the patient's parasite load and enhancing the efficacy of specific antiparasitic agents (Benaim et al., submitted). Finally, we have recently found that a group of bisphosphonates, non-hydrolysable analogs of PPi that are selectively accumulated in the parasite's acidocalcisomes inhibit *T. cruzi* hexokinase and up to 500 times more potent than PPi, the natural allosteric inhibitor of this essential enzyme. To assess which structural factors contribute to inhibitory activity, we determined the activity of 42 bisphosphonates against *T. cruzi* glycosomal hexokinase and the IC_{50} values obtained were then used to construct pharmacophore and comparative molecular similarity indices analysis (CoMSIA) models for enzyme inhibition. The pharmacophore models indicated the importance of hydrophobic and aromatic features together with two negative ionizable groups, suggesting binding to a large, hydrophobic site containing cationic residues or metal ions while CoMSIA field results showed the importance of electrostatic, hydrophobic as well as steric interactions (Hudock et al., submitted). −Julio A. Urbina, Ph.D. Laboratorio de Química Biológica, Centro de Bioquímica y Biofísica, Instituto Venezolano de Investigaciones Científicas Caracas, Venezuela International Research Scholar, Howard Hughes Medical Institute, Chevy Chase, Maryland, USA

[November, 2005-11-08 - 10h30 - ROOM B]

RT02C - PLANTS AS SOURCES OF NEW ANTILEISHMANIAL DRUGS

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A chalcone from *Piper aduncum* and flavonoids from *Kalanchoe pinnata* ("saião") were found to have potent antileishmanial activity in vivo. Whereas the chalcone act directly on the parasite inhibiting the sterol biosynthesis pathway, the *Kalanchoe* flavonoids act indirectly by activating the host iNOS enzyme and were shown to be effective in a human case of cutaneous leishmaniasis.

[November, 2005-11-08 - 10h30 - ROOM B]

RT02D - Protein Farnesyltransferase as a Target for *Trypanosoma brucei* Drug Development

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Background: Protein farnesyltransferase (PFT) catalyzes the addition of the 15-carbon farnesyl to the C-terminus of certain proteins. We have shown that PFT exists in trypanosomatid parasites. Our laboratory has screened a number of PFT inhibitor collections from industry and academia. **Methods**: *Trypanosoma brucei* (BF427) were used in screening assays. Tetrahydroquinoline (THQ) inhibitors were obtained from Bristol-Myers Squibb or were synthesized at University of Washington. PFT enzyme of *T. brucei* was made expressed in insect cells using recombinant techniques. **Results**: THQ compounds inhibit *T. brucei* PFTs with IC_{50}values as low as 0.5-1 nM. Cell growth inhibition (ED_{50}) was as low as 63 nM against T. brucei. Enzyme activity and anti-parasite activity were closely correlated. PFT inhibitors block farnesylation of proteins in growing parasites. Additional compounds have been synthesized that show improved pharmacokinetic properties compared to initial lead compounds. Murine experiments are in progress to establish the ability of THQ compounds to cure *T. brucei* infections *in vivo*. **Conclusion**: A series of tetrahydroquinoline inhibitors was shown to inhibit *T. brucei* PFT at low nanomolar concentrations, and to inhibit parasite growth with cidal activity. Chemical analogs are being made to optimize anti-parasitic activity and pharmacokinetic properties. Research supported by: Drugs for Neglected Diseases Initiative, Medicines for Malaria Venture, and NIH AI-054384.

[November, 2005-11-08 - 10h30 - ROOM C]

RT03A - Endosymbiosis in Trypanosomatids offer new clues on the origin of organelles

Cristina Motta *(UFRJ)*

Some trypanosomatids harbor a single obligate symbiotic bacterium in their cytoplasm. This endosymbiont divides in synchrony with the host protozoan, constituting a valuable model to understand the origin of organelles, such as the mitochondrion and the chloroplast. A mutualist symbiosis maintains both partners together, since the endosymbiont is unable to survive outside of the host, which is supplied with essential nutrients by the symbiotic bacterium, as hemin, aminoacids and vitamins. Like Gram-negative bacteria, the endosymbiont is enveloped by two unit membranes, however rather than a true bacterial cell wall, its peptidoglycan layer is reduced and modified. Differently from bacteria but similar to mitochondrion, the endosymbiont envelope lacks the septum and the FtsZ ring, a structure which plays an essential role in bacterial division. Lipid analyses of purified endosymbionts indicated a complete absence of sterols and a phospholipid composition markedly different from that found in the whole protozoan or in the mitochondrion. The major phospholipid found in the symbiotic bacterium was cardiolipin (CL), followed by phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Phosphatidylcholine is unusual for prokaryotes, except in those that maintain a close symbiotic or pathogenic relationship with eukaryotic cells, suggesting that the symbiont lipid biosynthetic pathways are relatively dependent from the host. Recent analysis showed that strains containing the endosymbiont display a two fold higher content of phospholipids, as compared to aposymbiotic strains. Cellular fractioning assays in *Crithidia deanei* grown in ^{32}P culture medium indicated that PC is the main phospholipid produced. Interestingly, the content of ^{32}P-PC

was 2 fold higher in mitochondrial fractions, as compared to that isolated endosymbionts. Previous studies revealed intensive metabolic exchanges between the endosymbiont and the host protozoan. Our results suggest that an intensive phospholipid trafficking occurs between the host trypanosomatid and its endosymbiont. Ongoing studies address the characterization of lipid metabolic pathways involved in this co-evolutionary process. Regarding the origin of the endosymbiont in the Trypanosomatidae family, 16S ribosomal DNA sequences indicated that the symbiotic bacterium is identical in different host trypanosomatid, being classified in the β division of Proteobacteria. The systematic sequencing of DNA from the endosymbiont reinforces this conclusion, indicating that this bacterium is closely related to *Bordetella bronchiseptica*. This suggests that a single event in nature gave rise to all endosymbiont-bearing trypanosomatids, recapitulating the process that led to the formation of mitochondrion in eukaryotic cells. Supported by: CNPq and FAPERJ

[November, 2005-11-08 - 10h30 - ROOM C]

RT03B - The evolution and diversity of insect kinetoplastid flagellates

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Our understanding of kinetoplastid evolution has improved within the last few years. Large taxon sampling of the 18S rRNA and SL RNA genes, and an increased application of protein-coding genes resulted in well-supported trees showing that trypanosomatids are monophyletic and are descended from within paraphyletic bodonids. Parasitism evolved at least four times in kinetoplastids. Analysis of molecular markers demonstrates that none of the monoxenic genera of trypanosomatids parasitizing insects are monophyletic. Several trypanosomatid species have low host specificity for insect hosts and some may even be able to infect warm-blooded vertebrates. A new trypanosomatid taxonomy that will deal with discrepancies between molecular phylogenetic data and cell morphology is needed.

[November, 2005-11-08 - 10h30 - ROOM C]

RT03C - Cell differentiation of "low" trypanosomatids: what's up?

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One of the most striking differences between vertebrates and lower eukaryotic cells is that in vertebrates, during embryogenesis, cells become progressively and irreversibly committed to specific lines of development. These cells undergo sequences of self-perpetuating internal changes that distinguish them and their progeny from other cells. On the other hand, trypanosomatids go through their life cycles alternating between proliferative forms in a stable environment and non-proliferative forms adapted to new and different environments. In other words, these organisms change their morphology and physiology through a process of reversible cell transformation (1,2). Cell differentiation has been fairly well studied in *Trypanosoma brucei* (1), *T. cruzi* (2) and *Leishmania* spp (3). On the other hand, little is known about differentiation of monogenetic trypanosomatids. Even so, it has been reported the induction of differentiation of *Herpetomonas* spp by changes in the physical conditions, such as temperature (4) and radiation (5), as well as by the addition of several drugs (6-9). Nonetheless, the mechanisms underlying this process in trypanosomatid parasites are poorly understood. Platelet-activating factor (PAF), a phospholipid with potent and diverse physiological and pathophysiological actions, was described as a powerful inducer of cell differentiation in *Herpetomonas muscarum muscarum* and *T. cruzi* (10,11). Also, we have described one of the mechanisms by which the latter transformation takes place, showing that PAF activates casein kinase 2 (CK2), via protein kinase C (PKC), and that a cell permeable inhibitor of CK2 (DRB) suppressed PAF-induced cell differentiation in a dose dependent manner (12). Additionally, we described the involvement of cyclic AMP (cAMP) in this process, showing that the enhancement of cellular differentiation promoted by PAF and cAMP in *H. m. muscarum* is mediated by a cAMP-dependent protein kinase (PKA). Lysophosphatidylcholine is an abundant component of plasma and oxidized low-density lipoprotein that displays several biological activities, some of which may occur through the PAF receptor. It has also been demonstrated that LPC is present in *Rhodnius prolixus* saliva, acting as an anti-haemostatic molecule (13). Interestingly, we have also described the effects of LPC on cell differentiation of *H. samuelpessoai*, which suggest that LPC modulates some important signaling pathways leading to cell differentiation of *H. samuelpessoai* and that phospholipase A2 probably rules this process by converting PC into LPC. Taken together, this set of results shed light on control mechanisms and their cooperation inside the living cell. Intracellular systems have many common features in multicellular and unicellular organisms, but also several differences that may lead to new targets for novel anti-parasitic drugs. References: (1) Gull, 2002, Curr Pharm Des. 8: 241-56. (2) De Souza, 2002, Kinetoplastid Biol. Dis. 1: 3-18. (3) Nugent et al., 2004, Mol Biochem Parasitol. 136: 51-62. (4) Roitman et al., 1976, J. Protozool. 23: 291-293. (5) Esteves et al., 1979, Acta Trop. 36: 257-266. (6) Angluster et al., 1977, J. Parasitol. 63: 922-924. (7) Thomas et al., 1981, Experim. Parasitol. 51: 366-372. (8) Castellanos et al., 1981, Acta Trop. 38: 29-37. (9) Lopes et al., 1983, Res. Commun. Chem. Pathol. Pharmacol. 42: 245-254. (10) Lopes et al., 1997, J. Euk. Microbiol. 44: 321-325. (11) Rodrigues et al., 1996, Biochem. Biophys. Res. Commun. 223: 735-740.

(12) Silva-Neto et al., 2002, Biochem. Biophys. Res. Commun. 293: 1358-1363. (13) Golodne et al., 2003, J. Biol. Chem. 278: 27766-27771. Supported by: CNPq, PIBIC-UFRJ/CNPq and FAPERJ. Cidade Universitária, Ilha do Fundão, Rio de Janeiro, R.J. 21941-590, Brasil. E-mail: angela.lopes@micro.ufrj.br

[November, 2005-11-08 - 10h30 - ROOM C]

RT03D - INTERACTION OF MONOXENIC TRYPANOSOMATID WITH VERTEBRATE AND INVERTEBRATE HOSTS.

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Infections by monoxenic trypanosomatids were described in HIV-1 positive patients, causing a leishmaniasis-like syndrome. These trypanosomatids, found mainly in insects, were normally non-pathogenic for mammals. With the emergency of monoxenic trypanosomatids as opportunistic pathogens in immunocompromised patients, we decided to study the co-infection of *Blastocrithidia culicis* and HIV-1, in vitro, as well as the interaction of this protozoan with the insect vector *Aedes aegypti*. Human monocyte-derived macrophages (MDM) were infected with a R5-tropic HIV-1 isolate using 10 ng/ml of p24 antigen. After 10 days infection, *B.culicis* was added to the HIV-infected MDMs at a 5:1 ratio, and cultures were kept for 48h at 35°C. Viral and parasite replication were evaluated by p24 ELISA assay and endocytic index, respectively. *B.culicis*-infected macrophages were also exposed to the HIV-1 transactivator protein (Tat), and protozoa growth was evaluated. We found that HIV-1 infection increased 4 times the *B.culicis* survival in MDM. Treatment with 100 ng/ml of HIV-1 Tat protein increased the protozoan growth 8 times after 24h, and 4 times after 48h, relative to control. Since Tat stimulates the production of TGF-β1 by macrophages, we also investigated the role of this cytokine in *B.culicis* infected-macrophages. Addition of TGF-β1 increased 3 to 7 times the endocytic index after 24h infection, and 4 times after 48h, relative to control. Ultrastructural analysis revealed that B.culicis not only survives in HIV-infected macrophages, but is also able to divide. Our results show for the first time that HIV-1 infection changes an otherwise non-pathogenic *B.culicis* to an opportunistic pathogen and this mechanism could be mediated by HIV-1 Tat protein through TGF-β1 production. As we were also interested in the transmission of these protozoa to the vertebrate host, we investigated if *B.culicis* was able to colonize *A. aegypti*, an important vector of human diseases such as dengue, yellow fever, filariasis and malaria. Our results demonstrated that *B. culicis* was able not only to adhere to *A. aegypti* midgut but also, to live and multiply in this mosquito for at least 48 days after feeding. Optical microscopy analysis revealed an infection process characterized by a homogenous distribution of the trypanosomatid along the epithelium of the digestive tract. No preferential interaction of protozoa with any cell type was observed. Ultrastructural analysis showed that during the colonization process, the protozoa interacted mainly with midgut cells through its flagellum, which penetrates the microvilli preferentially near the tight junctions. In order to characterize receptor(s) responsible for the protozoa recognition we resolved total midgut proteins by SDS-PAGE followed by Western blot, identifying a 29 KDa band able to bind biotinylated-*B.culicis*. Previous results from our group demonstrated that prolonged infections promoted insect midgut degradation which resulted in loss of microvilli and tight junctions of epithelial cells, allowing *B.culicis* to reach the mosquito haemocele. This suggested us the possibility of the protozoa interaction with salivary glands, similar to what happens in malaria. To address this question we incubated excised salivary glands of *A. aegypti* females with *B.culicis in vitro*. Our results demonstrated that this protozoan is able to bind to the salivary glands; logarithmic-phase protozoan bind significantly more than the stationary-phase. By demonstrating *B.culicis* colonization in a blood-sucking insect, we suggest that vector transmission of monoxenic trypanosomatids to vertebrate host may occur in nature. Supported by: CNPq, PAPES/Fiocruz, CAPES, Faperj, NIH-AIDS Research and Reference Reagent Program.

[November, 2005-11-08 - 10h30 - ROOM D]

RT04A - Switch it on, Switch it off: Ectopic gene regulation systems and their employment in Apicomplexan parasites

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Regulation of gene expression is crucial for all organisms in order to adapt to environmental changes. Although many stage-specific genes have been described in Apicomplexa our knowledge on the molecular mechanisms, especially the interplay between *cis-* and *trans*-acting elements remains poorly understood. Employing a genetic screen based on random integration mutagenesis we were able to identify two artificial transactivation domains in *Toxoplasma gondii* that are also functional in *Plasmodium falciparum* and allowed the establishment of Tetracycline-inducible gene expression systems in both parasites. These systems will be a valuable new tool for the characterisation of essential genes in these parasites. In contrast the identified transactivtion domains appear to be non-functional in higher eukaryotes, since no transactivation can be observed in HeLa-cells, suggesting substantial differences in the makeup of the transcription machinery between Apicomplexan parasites and their host. In addition we will discuss different strategies in order to generate conditional mutants using the currently available Tet-inducible systems in *P.falciparum* and *T.gondii*. Novel data on the characterisation of essential genes in *T.gondii* and *P.falciparum* using targeted disruption of candidate genes via homologous

recombination and a novel genetic screen based on random mutagenesis will be presented. Using these complementary strategies we intend to establish a library of inducible conditional mutants in order to identify novel essential genes that might represent novel vaccine and drug candidates against Apicomplexan parasites.

[November, 2005-11-08 - 10h30 - ROOM D]

RT04B - POSSIBLE IMPLICATION OF THE RHOPTRY PROTEIN RSP2 IN SEVERE P. FALCIPARUM ANEMIA

Jürg Gysin *(Unité de Parasitologie Expérimentale Institut Pasteur/Univ-Med. EA 3282)*

Anemia is undoubtedly one of the most common complications of P. falciparum infection and is particularly severe in children and pregnant women living in endemic areas. Its causes are multifactorial and several studies have shown that anemia results from the destruction of both infected (IEs) and uninfected erythrocytes (uEs) and is exacerbated by defects in erythropoiesis. Extravascular hemolysis of IEs and uEs occurs in hyperparasitemic infections and is thought to result from the destruction of erythrocytes by hemolysis and/or phagocytosis. The rigidification of IE and uE membranes may also be an aggravating factor, leading to the the destruction of these cells during their passage through the spleen. We recently showed that the rhoptry-associated ring surface protein 2 (RSP-2), unlike other parasite antigens, is present on the surface of IEs and uEs and is encoded by the RSP2/RAP2 gene, which is highly conserved in all known P. falciparum isolates. Merozoites transfer RSP2 to the erythrocyte surface during disturbed or aborted invasion, resulting in a transient (16-20 h) coating of some ring-stage IEs and up to 20% of uEs, depending on parasitemia. Similar results were obtained with various strains, but not with D10?RAP1 IEs (with a disrupted RAP1), which do not export RSP2 to the cell surface. Fluorescence recovery after photobleaching (FRAP) analysis of the mobility of RSP2 in the membrane revealed that RSP2 gradually spread from the merozoite contact site over the entire erythrocyte surface by a slow, lateral movement, in both IEs and uEs. Like other P. falciparum surface antigens, RSP2 may increase erythrocyte membrane rigidity, resulting in the destruction of these cells when they cross the interendothelial slits of venous sinus walls in the spleen, by mechanical retention followed by direct phagocytosis, opso-phagocytosis and pitting. Hosts respond to infection by increasing the proliferation and activity of macrophages responsible for the phagocytosis of IEs and uEs. A cytophilic (IgG2a) anti-RSP2 mouse mab, B4, promotes ERSP2+ phagocytosis and activates complement, as do sera from P. falciparum-infected anemic patients. Anti-RSP2 antibodies reactive with RSP2 molecules from various isolates are found in most individuals, including P. falciparum-infected pregnant women. We investigated whether the dyserythropoiesis frequently observed in anemic patients also involved RSP2 by culturing late-stage IEs with two erythroblast lines: HEL 92.1.7. and KMO-2. RSP2 was transferred by merozoites to the surface of both lines. Liquid IFA of fresh bone marrow samples from P. falciparum-infected anemic patients showed that there were up to 20% RSP2+ erythroid precursor cells, depending on parasitemia. It remains unclear whether RSP2-antibody-complement complexes on the surface of erythroblasts in vitro are responsible for this decline in precursor cells by phagocytosis, or whether these complexes are involved in the morphological alterations observed in the erythroid cells present in the bone marrow of malaria patients. It has been suggested that, in some conditions, activated bone marrow macrophages may damage precursor cells. Under our experimental conditions, we observed no phagocytosis or apoptosis. A large percentage of RSP2+ erythroblasts were found to be dead after 24 to 48 hours of coculture with mab B4 and adherent human monocytes. This was not the case if mab B4 was replaced by a negative isotypic control or a non cytophilic anti-RSP2 mab. In conclusion, we have identified a novel molecular mechanism of host-parasite interaction that may set the conditions for the destruction of IEs and uEs as precursor cells of the erythroid lineage in malaria patients. This discovery sheds light on the possible mechanisms of malarial anemia. Epidemiological studies are now required to determine the extent of insertion of critical regions of RSP2 in the membrane of erythroid cells.

[November, 2005-11-08 - 10h30 - ROOM D]

RT04C - Variant surface antigens and immunity to *Plasmodium falciparum* malaria

Lars Hviid *(Rigshospitalet-DK)*

People living in areas of intense and stable transmission of *P. falciparum* parasites acquire immunity to malaria during childhood, but protection is only acquired after several years and many disease episodes. However, the facts that acquisition of substantial protection against some severe malaria syndromes can be fairly rapid following natural parasite exposure and that short-lasting sterile protection has be achieved with some experimental vaccines have raised hopes that the development of effective vaccines against this major cause of human misery is a realistic goal. However, the uncertainty regarding the antigenic targets of naturally acquired protective immunity and the immunological mechanisms involved, and the apparent need for continuous antigenic challenge to maintain acquired protection, remain major vaccine development obstacles. A coherent theoretical framework of how protective immunity to *P. falciparum* malaria is acquired following natural exposure to the parasites is now beginning to emerge, not least thanks to studies that have combined clinical and epidemiological data with basic research in the immunology, molecular biology, and parasitology of the disease. These studies strongly indicate that naturally acquired protective immunity is mainly mediated by IgG, and that the main target for these antibodies is the clonally variant antigens that the parasites expose on the surface of the infected erythrocytes. This framework can resolve many of the difficulties in relating particular immune responses with

clinical protection, not least the long-standing conundrum of the re-emergence of disease susceptibility in previously immune women when they become pregnant. Furthermore, it suggests that a radically new approach to malaria control based on targeted control of syndrome-specific morbidity and mortality by vaccination is a realistic goal that is highly suitable for vaccination against *P. falciparum* malaria in endemic areas.

[November, 2005-11-08 - 10h30 - ROOM D]

RT04D - Extense variant gene family repertoire overlap in Western Amazonian *Plasmodium falciparum* Isolates

Letusa Albrecht *(Instituto de Ciencias Biomédicas)*; Fernando Merino *(Instituto de Ciencias Biomédicas)*; Erika H. E. Hoffmann *(Instituto de Ciencias Biomédicas)*; Marcelo U. Ferreira *(Instituto de Ciencias Biomédicas)*; Ricardo G. M. Ferreira *(Instituto de Ciencias Biomédicas)*; Ana Lúcia Osakabe *(Instituto de Ciencias Biomédicas)*; Alan M. Durham *(Instituto de Matemática e Estatística)*; Hernando A. del Portillo *(Instituto de Ciencias Biomédicas)* Gerhard Wunderlich *(Instituto de Ciencias Biomédicas)*

In order to find a molecular basis for observations describing relatively fast developing immunity to malarial infection in the Western Amazon region, the *var*, *stevor* and *rif* gene repertoire of nine different *Plasmodium falciparum* clones collected in 1985 and 2000-2004 was evaluated. In contrast to previous results from South East Asia, the variant gene repertoire in Brazilian *P. falciparum* isolates appears rather small and redundant. While the individual repertoire size of Brazilian strains did not differ from South East Asian isolates, we found a three times higher overlap of the *var* gene repertoires in Amazonian strains which was also conserved over time, suggesting the ongoing circulation of a limited *var* gene repertoire. Additionally, more than 50% of the 106 different sequences identified herein showed the highest degree of similarity to *var* genes from either Brazilian or Venezuelan isolates, indicating a limited genetic pool of Plasmodium falciparum in the Amazon Basin as a whole. Despite of higher copy numbers per genome, *rif* genes also showed a significant repertoire overlap comparable to that of *var* genes. *Stevor* genes, which share the same predominant subtelomeric localization as *var* and *rif* genes, showed a still higher repertoire overlap and very high similarity to 3D7 *stevor* genes, indicating stronger functional conservation than *var* and *rif* genes. This is the first study that reveals i) that variant *Plasmodium falciparum* gene repertoires of certain areas can be limited and ii) that *stevor* genes probably do not function as mediators of the immune escape in the same manner as *var* or *rif* genes and iii) that the global *rif* -gene family is immense. Supported by FAPESP

[November, 2005-11-08 - 14h00 - ROOM A]

RT05A - ROLE OF CHAGASIN AS A MODULATOR OF THE MATURATION AND SORTING OF CYSTEINE PROTEASES IN *Trypanosoma cruzi*

Ana Paula C.A. Lima *(Universidade Federal do Rio de Janeiro)*

In the last decade, clan CA cysteine proteases belonging to the papain superfamily have been validated as promising targets for alternative anti-trypanosome drugs. Genes encoding cathepsin L-like and cathepsin B-like cysteine peptidases have been found in the genomes of the three Tryps, and the former are grouped in large polymorphic gene families. In *T. cruzi*, the term cruzipain refers to a group of iso-enzymes presenting dual cathepsinL-like and cathepsin-B like specificity, which may be differentially expressed depending on the parasite life stage or strain. These molecules are synthesized as inactive precursors that require proteolytic excision of the N-terminal pro domain in order to become active, an event thought to occur by auto-catalytic processing. Clues to understand the role of cruzipains in *T. cruzi* emerged mainly from studies using synthetic irreversible inhibitors *in vitro*, which prevent amastigote survival/replication in the cytoplasm of infected cells and block, at least partially, the invasion of different cell types by trypomastigotes. Exposure of epimastigotes to these compounds leads to disruption of the intracellular traffic, presumably due to accumulation of unprocessed cruzipain precursors in the secretory pathway. This phenomenon results in the enlargement of Golgi cisternae, ultimately provoking drastic abnormalities in the secretory pathway and parasite death. Thus, the maintenance of pro-cruzipain maturation at constant rates seems crucial to keep intracellular traffic functioning adequately. Recently, we have identified a new type of cysteine peptidase inhibitor in *T. cruzi*, which was named chagasin. Chagasin is a 11 kDa protein encoded by a single gene, whose homologues are present in other trypanosomatids and in bacteria. We have recently shown that chagasin is able to interact with cruzipain *in vivo*, forming tight binding complexes and regulating the activity of the enzyme. Genetically modified *T. cruzi* epimastigotes expressing 4-fold more chagasin have significant less cruzipain activity, display lower capability to differentiate into trypomastigotes and are more resistant to a synthetic cysteine protease inhibitor, as compared to the wild-type. Tissue culture trypomastigotes are less infective than wild type *in vitro*, due to lower membrane-associated cysteine protease activity. Since pro-cruzipain maturation is thought to occur by auto-catalysis, we asked if chagasin may contribute to the control of zymogen processing in epimastigotes. The Golgi complex was identified as the site of pro-cruzipain localization by fluorescence microscopy using antibodies raised against the recombinant pro domain of cruzipain. In parasites overexpressing chagasin, we observed higher contents of pro-cruzipain and increased secretion of these precursors to the flagellar pocket. Sub-cellular fractionation followed by biochemical and ultra-structural characterization of the endo-lysosomal compartments allowed the identification of two distinct sub-populations of reservosomes, based on differential density (R1 and R2). Mature cruzipain accumu-

lates preferentially in the R1 sub-population while chagasin is equally distributed in both sub-populations. In epimastigotes overexpressing chagasin, cruzipain activity is marked decreased in R1, while there is no alteration in the chagasin contents in this sub-population, suggesting that the reduction in mature cruzipain in R1 results from altered traffic of the protease to these organelles. The dynamics of intracellular traffic in epimastigotes was evaluated by analyzing endocytosis of fluorescent tracers. Both the internalization of transferrin-FITC and the subsequent recycling of the fluorofore to the extracellular milieu were slower in chagasin overexpressing parasites. However, there was no detectable alteration in the internalization of BSA-FITC. We propose that chagasin contributes to the control of pro-cruzipain activation and sorting, ultimately influencing the traffic in the secretory compartments.

[November, 2005-11-08 - 14h00 - ROOM A]

RT05B - Trafficking of cruzain, the major cysteine protease of Trypanosoma cruzi

HUETE-PEREZ,J.A. *(University of California San Francisco)*; ENGEL,J.C. *(University of California San Francisco)*; MCKERROW,J.H *(Universidad Centroamericana)*

Cruzain (cruzipain) is the major cysteine protease of Trypanosoma cruzi, the causative agent of Chagas' disease. Previous work in our laboratory established that the effect of inhibitors on T. cruzi parasites was due to inhibition of cysteine protease precursor processing resulting in accumulation of a parasite protease precursor in the organism's Golgi apparatus, resulting in osmotic shock to the organelle system of the parasite and, ultimately, death (Ref 1). We also identified the pro-domain of cruzain as a key element in trafficking of the protein to the lysosomal compartment (Ref 2). Currently we are studying the intracellular trafficking of the cruzain enzyme with the aim of identifying potential interacting protein molecules that might be exploited for chemotherapy. In this paper, we present an overview of our current knowledge of cellular targeting mechanisms in T. cruzi, emphasizing on our data related to the identification of interacting molecules. (Ref 1) Engel JC, Doyle PS, Palmer J, Hsieh I, Bainton DF, McKerrow JH. Cysteine protease inhibitors alter Golgi complex ultrastructure and function in Trypanosoma cruzi. Cell Sci 1998 Mar; 111:597-606. (Ref 2) Huete-Perez JA and McKerrow JH. Intracellular protease trafficking in kinetoplastids is mediated by the prodomain.

[November, 2005-11-08 - 14h00 - ROOM A]

RT05C - Cysteine Proteases of *Giardia lamblia*

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Giardia is an important cell biology model because it represents the most basal of eukaryotic lineages [1]. *Giardia* trophozoites lack many typical eukaryotic organelles such as mitochondria, peroxisomes, and protein trafficking components such as a classical Golgi apparatus and secretory granules [2]. Instead, *Giardia* employs a simple endomembrane system to export proteins to distinct intracellular compartments. In the replicative trophozoite stage these compartments include an extensive tubulovesicular network with ER-like structure (decorated with ribosomes) and acidified peripheral vacuoles [3]. Resident proteins are found to function within each of these locations. *Giardia* contains a gene family of cysteine proteases homologous to cathepsin L and cathepsin B found in lysosomes of higher eukaryotic cells. Utilizing reporter-constructs, confocal microscopy, and ultrastructural analysis, we have shown that cathepsin B-like proteases are primarily localized in a tubulovesicular network, peripheral to the perinuclear membrane, and having ultrastructural and biochemical characteristics of ER. Concurrent analysis of uptake of labeled proteins demonstrates very rapid endocytosis into the same compartment where the endocytosed proteins are degraded by the *Giardia* homologs of lysosomal cathepsins. It appears therefore that *Giardia* contains a transitional endomembranous structure that may pre-date compartmentalization of endocytic/ lysosomal functions and the endoplasmic reticulum. Characterization of this primitive endomembrane system may provide clues that lead to a better understanding of the evolution of cell compartmentalization. 1. Sogin ML, et al. Science 243:75-77, 1989. 2. Adam RD. Clin. Microbiol. Rev. 14:447-475, 2001. 3. Soltys BJ, Falah M, Gupta RS. J Cell Sci. 109:1909-17, 1996.

[November, 2005-11-08 - 14h00 - ROOM A]

RT05D - Dibucaine effects on the endocytic/exocytic pathways on *Trypanosoma cruzi*

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In spite of the large amount of studies about local anesthetics, the mode of action of these substances is not well understood. There are two hypotheses to explain the action of local anesthetics (LA). One of them is based on a non-specific perturbation of the lipid bilayer structure and is called the Lipid Hypothesis. The second one, the Protein Hypothesis, is based on evidences of specific interactions of LA with distinct classes of proteins such as cell membrane receptors, enzymes and cytoskeleton proteins (1-3). Local anesthetics such as quinacrine and dibucaine were found to inhibit the activation of a cytoplasmic calcium independent phopspholipase A_2 ($Ipla_2$) resulting on a significant inhibition of intracellular membrane-bound compartments fusion

along the endocytic and secretory pathways (4-6). The use of substances that interfere with homotypic and heterotypic fusions in the endocytic/exocytic pathways can be used as a new approach to understand the nature of distinct compartments mainly in cells where they have not been properly identified yet. The effect of dibucaine on protein internalization in epimastigote forms of *T. cruzi*, was assayed by flow cytometry. Fluid phase and receptor-mediated endocytosis were evaluated after incubation of parasites in the presence of fluorescent-labeled BSA and non-related IgG for 30 min to 4 h in the presence or absence of Db. Dibucaine inhibited the endocytosis of both tracers with inhibition rates of about 70 % after 2 h incubation. In parasites submitted to a 4 h pulse and 20 h chase in the presence of gold-labeled BSA, we observed that there was no apparent delay on the labeling of the different compartments and that the drug did not impair the fusion of pre-labeled and new-labeled compartments as previously described for macrophages (2). Due to the natural acidic reservosome environment and the action of its enzymes, protein-gold complexes were naturally degraded and the gold particles precipitated forming large aggregates. In dibucaine treated parasites however, those aggregates were not observed suggesting that the degradative process of protein were not occurring. One of the hypotheses to explain those results is that dibucain could be causing an alteration on the pH of reservosomes compartments. However, control and treated parasites showed no differences in the pH of reservosomes. The other possibilities were, a direct action of dibucaine on the proteolytic activities of the parasite, the inhibition of expression of cruzipain (CP) the major cysteine protease of *T. cruzi* and the subversion of the regular traffic of hydrolases. To investigate whether proteases were affected by Db their activities were assayed on SDS gels using gelatin as substrate, which showed that were no differences between control and treated parasites. Dibucaine induced a slightly reduction in the cysteine protease activity observed in the cellular extract and in the supernatant of epimastigotes culture. The immunocytochemical detection of cruzipain revealed that Db induced an accumulation of CP on the cell surface of epimastigote forms. REFERENCES 1- Hollmann, M.W., Wieczorek, K.S., Berger, A. and Durieux, M.E. Mol. Pharmacol. 59: 294-301, 2001. 2- Mayorga, L.S., Colombo, M.I., Lennartz, M., Brown, E.J., Arman, K.H., Weiss R., Lennon, P.J. and Stahl, P.D. Proc. Natl. Acad. Sci. USA 90:10255-10259, 1993. 3- Mondal, M. and Chakrabarti, A. FEBS Lett. 532: 396-400, 2002. 4- De Figueiredo, P., Drecjtrah, D., Katzenellenbogen, J.A., Strang, M. and Brown, W.J. Proc. Natl. Acad. Sci. USA 95: 8642-8647, 1998. 5- De Figueiredo, P., Drecjtrah, D., Polizotto, R.S., Cole, N.B., Lippincott-Schwartz, J. and Brown, W.J. Traffic 1: 504-511, 2000. 6- Lennartz, M.R., Yuen, a.F.C., Masi, S.M., Russel, D.G., Buttle, K.F. and Smith, J.J. J.Cell Sci. 110: 2041-2052, 1997.

[November, 2005-11-08 - 14h00 - ROOM B]

RT06A - Pharmaceutical Nanotechnology: a tool for designing anti-infective strategies.

Romero,E.L. *(Laboratorio de Diseño de estrategias de Targeting de Drogas (LDTD))*

Up to the present, the experimental chemotherapeutic strategies assayed against the Chagas disease are scarce and have not produced encouraging results. One of the main unsolved challenges is the finding of a selective and efficient medication capable of eliminating the *Trypanosoma cruzi* nests in the cytoplasm of infected cells. Likewise, and several steps ahead in the scale of technical difficulties, the feasibility of counting on with a vaccine against this disabling, often deadly and endemic in several South American countries disease, is still utopian. In this context, we will describe our experience of using Nanotechnology as a tool for designing *nanodevices* or *nanoships* that enable a controlled space-temporal delivery of different molecules with anti-infective activity, be it to tissues, organs, cells or intracellular compartments where the parasites hide. Only Nanotechnology allows the bottom-up design of those *nanoships* capable of triggering drug delivery in response to determined external stimuli. Different self assembled *nanoships* with a size 5-100 nm can be fabricated in several shapes and degree of complexity, starting from small molecular blocks extracted from natural, cheap and sustainable sources. Those molecules with pharmacokinetics and biodistribution drastically modified upon being loaded on the *nanoships*, can exert therapeutic effect even administered at hundred times less concentration than conventional medication, and with less toxicity. Those facts make possible to develop anti-infective strategies in two fields: the chemotherapeutic, by achieving selective and massive delivery of therapeutic molecules to a particular diseased target; and the immunological, by generating adjuvancy with the adequate response profile in order to elicit protection. We will show our ongoing research, first on the effect on experimental parasitemia of mice treated with pH-sensitive *nanoships* that once in the endosomal compartment of infected cells massively deliver the anti-infective drug straight to the cytoplasmic nests. Second, we will briefly survey the preliminary results in terms of immune response against a model protein loaded in *nanoships*, capable of eliciting adjuvancy, determined by humoral and cellular response, as well as memory after 200 days post initial administration. Very importantly, these last *nanoships* are not only capable of inducing adjuvancy several orders of magnitude higher that current *cpg* motifs - strategies, but also due to their special structure they could be envisioned as suitable candidates for oral vaccination. Supported by Secretaria de Investigaciones de la Universidad Nacional de Quilmes, Buenos Aires, Argentina.

[November, 2005-11-08 - 14h00 - ROOM B]

RT06B - Discovery of new drugs for diseases of the developing world

Federico Gomez de las Heras *(GlaxoSmithkline, Madrid Spain)*

The GlaxoSmithkline Disease of the developing World Drug Discovery Centre at Tres Cantos, Madrid, Spain is focused on the discovery of development candidates for the treatment of malaria and tuberculosis. This effort is done in partnership with Medicines for Malaria Venture (MMV) and Global Alliance for TB (GATB). This presentation will review the discovery process of drugs for Diseases of the Developing World, and will focus on the discovery of pyridones, a new class of antimalarial drugs acting by inhibition of plasmodia electron transport.

[November, 2005-11-08 - 14h00 - ROOM C]

RT07A - Analysis of the multigene families of mucins and U-rich RNA Binding Proteins in *Trypanosoma cruzi*

Campo, V. *(USM-ARG)*

Trypanosoma cruzi, the causative agent of Chagas' disease have to deal with enviromental changes during the interaction with both insect and mammalian hosts. Pivotal for its success is the continuous remodeling of the surface coat, which allows the adaptation to the different environments faced during its complicated life cycle. The surface of *T. cruzi* is covered in mucins that contribute to the parasite protection and to the establishment of a persistent infection. Mucins are basically core polypeptides displaying an extensive glycosylation that impose an extended conformation to the molecule, which is often used to elevate the outmost domains above the cellular glycocalyx thus facilitating their specific interaction with receptors and/or lectins. Their importance in *T. cruzi* is underscored by the presence of ∼850 genes comprising ∼1% of the parasite genome. These genes have been classified in two main multigenic families differentially expressed during the life cycle. The coordinate expression of a large repertoire of mucins exhibiting variable regions is distinctive to the mammal-dwelling stages, thus suggesting a strategy to miscarry the host immune response. In contrast, in the insect host only mucins with no variable regions are exposed on the parasite surface. The expression of this last mucin family showed to be controlled by a post-transcriptional regulatory mechanism, characteristic of most kinetoplastid parasites. The turnover of these mucin transcripts is controlled by an AU-rich element localized in the 3'-untranslated region wich is recognized by RNA-binding proteins. The analysis of the genes coding for these proteins led to the characterization of a family, named TcRBP for *T. cruzi* RNA-binding proteins, with six members identified so far, all sharing almost the same RNA-Recognition Motif (RRM) but different auxiliary domains. The combination of RRM domains with different auxiliary domains confers to these proteins a modular structure similar to the ones found in transcription factors. RRM-type proteins modulate transcript stability and translation efficiency, among other processes. Predicted proteins containing RRM domains were identified from the complete predicted proteomes of *T. brucei, T. cruzi* and *L. major*. 139 RRM-type proteins were found in *T. cruzi* with two or more paralogues, presumably because the CL-Brener clone used for sequencing has a hybrid genotype. The developmentally regulated expression of TcRBP members might be related with stage-specific mRNA turnover of specific transcripts and, thus, required for the survival of the parasite under different enviroments.

[November, 2005-11-08 - 14h00 - ROOM C]

RT07B - Genomic sequence variability and DNA Repair in Trypanosoma cruzi

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T. cruzi has an heterogeneous population, composed of a pool of strains presenting distinct characteristics. In spite of this broad diversity, two major lineages of the parasite, named T. cruzi I and T. cruzi II, have been identified. These divergent lineages occupy distinct ecological environments: the silvatic cycle (T. cruzi I) and domestic cycle (T. cruzi II) of Chagas disease. In order to understand the mechanisms involved in the generation of genetic variability in the T. cruzi population, we began to study genes involved with DNA recombination and repair in the parasite. The T. cruzi MSH2 gene, whose product is an essential component of the mismatch repair (MMR) machinery, was cloned and its sequence determined in several strains. Single-nucleotide polymorphisms (SNPs) analyses of the TcMSH2 identified three haplogroups: two haplogroups, denominated A and C, present a strong correlation with the previously described T. cruzi lineages I and II, respectively, and a third haplogroup (B) comprising strains presenting hybrid characteristics, among them the CL Brener clone. All strains from a specific TcMSH2 haplogroup encoded the same protein isoform, and more importantly, the sequence differences between the MSH2 isoforms seem to correlate with variations in the efficiency of mismatch repair in the parasite. Cultures of Esmeraldo cl3 and JG strains (both belonging to haplogroup C) were more resistant to cisplatin treatment than Silvio X-10 cl1 and Col1.7G2 (belonging to haplogroup A), a characteristic known to be conferred by a deficiency in mismatch repair. Conversely, survival rates were significantly higher in Silvio

X-10 cl1 and Col1.7G2 cultures (haplogroup A) in the presence of H2O2 than in Esmeraldo cl3 and JG strains. Analyses of microsatellite loci showed no evidence of microsatellite instability in strains representative of haplogroup A, but new microsatellite alleles were definitely seen in strains from haplogroups B and C cultured in the presence of hidrogen peroxide. Altogether, our data suggest that strains belonging to haplogroups B and C (T. cruzi II) have decreased mismatch repair ability when compared to haplogroup A (T. cruzi I) strains. We then decided to investigate whether the metabolic differences in the MMR pathway could be reflected in the antigenic diversity of the parasite population. Analysis of DNA sequences derived from two multi-gene families encoding an RNA binding protein (the TcRBP48 antigen) and the amastin surface protein corroborates this hypothesis. Analyses of PCR-amplified genomic fragments derived from six strains showed that the sequence variation found in these samples correlated well with the division of T. cruzi into three haplogroups. Most importantly, both multicopy gene families were less variable in strains belonging to MSH2 haplogroup A (T. cruzi I) when compared with sequences from haplogroup B and C (T. cruzi II and hybrid strains). To further investigate the role of MSH2 in the generation of variability in T. cruzi, we developed parasite knock-outs for the MSH2 gene. Stable lineages derived from the clone CL Brener (which presents MSH2 alleles corresponding to haplogroups B and C) with one deleted allele (msh2+/-) were assessed for survival in response to genotoxic agents. Consistent with the role of MSH2 in MMR, CL Brener msh2+/- clones presented a ~40% lower survival to H2O2 than wild type cultures. We are currently characterizing double knock-out mutants which will be used in experiments designed to test the MMR activity in transfected parasites expressing each one of the three distinct MSH2 isoforms.

[November, 2005-11-08 - 14h00 - ROOM C]

RT07C - Characterization of CCCH zinc finger proteins of *Trypanosoma cruzi*

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Gene expression in *Trypanosoma cruzi* is mainly regulated by post transcriptional mechanisms involving the control of mRNA stability and translation. Hence, in addition to the general mRNA decay machinery this parasite must have stage-specific mRNA decay pathways allowing the modulation of its gene expression pattern. The gene regulation mechanisms acting on mRNA stability are probably critical for *T.cruzi*, as the parasite is able to adapt rapidly to changes in environmental conditions. The stability of many developmentally regulated transcripts in differentiating trypanosomatids appears to be determined, at least in part, by structural elements and specific cis-acting sequences within the 3-UTR of the mRNAs, which function as either regulatory or cleavage targets. In recent years, research has focused on the identification of the trans-acting factors that interact with sequence motifs in mRNAs and modulate translational efficiency or mRNA stability in kinetoplastida. Recently, proteins containing a CCCH zinc finger motif C(X8)C(X5)C(X3)H, a characteristic feature of some RNA-binding proteins, were characterized in *T. cruzi* and *T. brucei.* At least one of these proteins (TbZFP1) has been shown to be involved in the control of *T. brucei* differentiation to the procyclic form. CCCH zinc finger proteins are heterogeneous regarding the number of their CCCH motifs and biological function, such as ensuring RNA stability, distribution or translation and RNA processing. Recently, tristetraprolin, a member of a small family of proteins bearing two copies of the unusual CCCH zinc finger domain, was found to destabilize ARE-containing mRNAs, such as cytokine and growth factor mRNAs. We reported the characterization of the *T. cruzi* homolog of tbZFP1, named tcZFP1. Unexpectedly, this protein recognizes C-rich ribopolymers in vitro instead of ARE motifs. The tcZFP1 gene is differentially expressed throughout metacyclogenesis. Hence, it might be involved in modulating stage-specific mRNA decay pathways in *T. cruzi.* Preliminary analysis of the proteins associated to the polysomes of *T. cruzi* by mass spectrometry have identified two novel CCCH proteins, suggesting that some of them can modulate the accessibility of mRNAs to the translation machinery, a mechanism that is ultimately involved in the differentiation of infective parasite stages. The complete genome sequencing of *T. cruzi*, *T. brucei* and *Leishmania major* has identified a large number of genes encoding putative CCCH zinc finger proteins. In *T. cruzi* the number of such genes is around 80. A more detailed analysis shows that these genes can be grouped in alleles and encode potentially 39 distinct CCCH proteins. Most of the TcZFP genes have orthologs in *T. brucei* and *Leishmania*, suggesting that CCCH proteins could be key proteins in modulating the RNA life in all trypanosomatids. Out of TcZFP1, nothing is known about the cis-acting elements within the RNA molecules involved in the putative interaction with CCCH zinc finger proteins in trypanosomatids. For this reason, we used EMSA to define the potential sequence targets of other TcZFPs in vitro. We are currently carrying out overexpression experiments in conjunction with microarray analysis to provide new insights into the expression patterns of the genes encoding CCCH zinc finger proteins in *T. cruzi.*

[November, 2005-11-08 - 14h00 - ROOM C]

RT07E - Translation initiation in Trypanosomatids: preliminary characterization of multiple homologues for the eIF4F subunits and the poly (A) binding protein (PABP).

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In Trypanosomatids the lack of transcriptional control for the synthesis of the mRNA coding genes has implicated the initiation stage of protein synthesis, or translation, as a likely major step for regulation of gene expression. However, little is known about protein synthesis in these pathogenic protozoans. In mammals, yeast and plants translation initiates with the binding of the heterotrimeric translation initiation complex eIF4F-eIF4E, eIF4A and eIF4G-to the monomethylated cap present on the 5‘nd of mRNAs. Subsequently eIF4F recruits the small ribosomal subunit which then scans the mRNA 5‘UTR until it finds the translation initiation codon and starts protein synthesis. The eIF4E subunit is the cap binding protein which interacts directly with the 5‘cap nucleotide, eIF4A is a highly conserved RNA helicase and eIF4G acts as a scaffold for the complex with binding sites for both eIF4E, eIF4A and other translation factors. eIF4G also binds to the poly(A) binding protein (PABP) bound to the 3‘end poly(A) tail of the mRNA, causing its circularisation. Our work focuses on the characterization in Trypanosomatids of homologues to the various eIF4F subunits as well as PABP. We have identified several potential eIF4F and PABP homologues in *Leishmania major*, *Trypanosoma brucei* and *T. cruzi* genome databases: 4 eIF4Es, 2 eIF4As, 5 eIF4Gs and 2 PABPs, all conserved and similar in sequence identity in the three organisms. A third PABP homologue was only found in *L. major*. Several of these eIF4F/PABP homologues from *L. major* and *T. brucei* were cloned and used for protein expression and antibody production. The recombinant polypeptides and antibodies were then used to study the various proteins biochemically. In vivo assays in *T. brucei* were also performed to further characterise the eIF4E and eIF4A homologues. So far we have been able to identify significant differences between the homologues in overall gene expression, subcellular localisation and RNA/protein binding affinities. The various eIF4Es (named as LmEIF4E1-4/TbEIF4E1-4 for the *L. major* and *T. brucei* homologues respectively) were seen to differ in their affinity to a synthetic mammalian cap and also in their binding to eIF4G. Differences in abundance of up to 50 fold were seen between some of the homologues in both *T. brucei* and *L. major*. Indeed, one of the four eIF4E homologues (TbEIF4E3) was not only seen to be essential for viability in *T. brucei* procyclic forms but its expression was stage specific since it is very abundant in procyclic and nearly absent from bloodstream forms. The two eIF4As (LmEIF4A1-2/TbEIF4A1-2) were seen to differ not only in abundance but, in *T. brucei* at least, also in subcellular localization, sensitivity to RNA interference and inhibition by a dominant negative mutant. It seems that only the 4A1 homologue is involved in translation and that 4A2 might be an orthologue of mammalian eIF4AIII, which is involved in various processes dealing with mRNA metabolism mainly in the nucleus. For the various eIF4Gs (LmEIF4G1-5/TbEIF4G1-5), preliminary evidence indicates significant differences in binding to the eIF4E, eIF4A and PABP homologues. The study with the PABP homologues (LmPABP1-3/TbPABP1-2) is just starting but already differences are seen between the first two homologues studied (LmPABP1-2) since only LmPABP1 is found in a phosphorylated form. So far most orthologues from *L. major* and *T. brucei* seem to share similar properties indicating conserved but unique roles in protein synthesis in these parasites. We discuss possible implications of our results in parasite biology and regulation of gene expression.

[November, 2005-11-09 - 10h30 - ROOM A]

RT08A - The diversity and peculiarity of Rab small GTPase in vesicular trafficking of the enteric protozoan parasite Entamoeba histolytica

Tomoyoshi Nozaki *(Gunma University Graduate School of Medicine)*

Vesicular (membrane) trafficking plays an indispensable role in the parasitism and pathogenesis of the protozoan parasite Entamoeba histolytica. Amebic trophozoites ingest and feed on microorganisms and mammalian cells. The amebae also secrete a number of degradative molecules including cysteine proteases and amoebapores responsible for the destruction of host tissues. Although recent completion of the Entamoeba genome shed light on the extreme complexity of membrane trafficking in this parasites (e.g., more than 90 Rab genes), molecules and molecular mechanisms involved in vesicular trafficking remain largely unknown. In this presentation our on-going studies to attempt to dissect molecular mechanisms of vesicular trafficking in this parasite will be presented. First, proteomic analysis of phagosomes revealed a panel of 170 proteins. We demonstrated dynamic changes of phagosome proteins during phagosome maturation, as well as variations of phagosome proteins between isolates. We also verified both presence and kinetics of representative phagosome proteins by immunoblot, immunofluorescence, and video microscopy. Second, we have established

video microscopy to monitor vesicular pH, degradation of ingested materials, and the movement of GFP-tagged amebic proteins (e.g., Rab or FYVE domain-containing proteins), in a living cell. We demonstrated, using the system, the dynamism of phagosome acidification and degradation of its content. Acidification of phagosomes occurred very rapidly (within 1.5-2 min) and persisted for 12 h, while degradation of ingested Leishmania promastigotes occurred within 20 min. We also observed remarkable differences in the kinetics of phagosome maturation between E. histolytica and its related and non-pathogenic species E. dispar and between highly virulent and avirulent strains of E. histolytica. Third, we identified and characterized a few Rab proteins, namely Rab5 and Rab7A, involved in the formation of unique preparatory vacuole called prephagosomal vacuole. In addition, we discovered a novel effector complex of Rab7A, which regulates targeting of hydrolases to phagosomes. These studies should provide molecular basis of pathogenesis of this important pathogen. Loftus, Nature 433, 865-868. Okada, Eukaryot. Cell 4, 827-831. Saito-Nakano, Exp. Parasitol. 110, 244-252. Saito-Nakano, J. Biol. Chem. 279, 49497-49507. Nakada-Tsukui, Mol. Biol. Cell in press.

[November, 2005-11-09 - 10h30 - ROOM A]

RT08B - A Parasite Strategy to Subvert Nutrients: The Garroting of Host Lysosomes by *Toxoplasma*

Isabelle Coppens *(JHSPH-)*

There are a limited number of strategies by which intracellular pathogens can divert host nutrients. Such mechanisms depend, in part, on the intracellular locale in which the organism resides in mammalian cells. For pathogens living freely in the host cytosol, diffusion and/or transport of host molecules across the microbial outer/plasma membranes, or endocytosis of macromolecules from the host cytosol can occur. For pathogens residing intracellularly in host endocytic compartments, fusion of host endocytic organelles with the pathogen vacuole can deliver contents to the vacuolar space, from where they can be taken up by the mechanisms described above. The most enigmatic situation exists with pathogens living in a vacuole which does not intersect the host cell endocytic pathway. One such organism is *Toxoplasma gondii*, a member of the apicomplexan family. *T. gondii* is adapted to thrive in a unique self-made niche, i.e. the parasitophorous vacuole (PV), within the cytoplasm of a large variety of mammalian cells. The PV is segregated from host cell endocytic or exocytic pathways, which negates the possibility of acquisition of proteins or lipids whose trafficking is restricted to those pathways. Unequivocally, we found that *T. gondii* is auxotrophic for cholesterol. The parasite is equipped to divert low density lipoprotein (LDL)-derived cholesterol through host LDL receptor-mediated endocytosis. The absence of fusion between host endocytic compartments and the PV suggests, speculatively, that LDL cholesterol might be transported through the host cell to the PV membrane by cholesterol transport proteins. From that destination, it has been suggested that an extensive collection of elongated nanotubules forming a tubulo-reticular network in the vacuolar space, might participate in delivering nutrients from the PV membrane to the parasite. Indeed, this membrane network appears fused at points with the PV membrane, providing a potential physical connection for lipid traffic from the PV membrane to the vacuolar space, and potentially to the parasite, or in reverse. We resolve this speculation, in unexpected fashion. We show that the PV of *T. gondii* can indeed accumulate material coming from the host cell via the exploitation of the host endo-lysosomal system. Host endocytic structures are translocated along host microtubules to the PV and delivered intact into the vacuolar space by microtubule-based invaginations of the PV membrane. The tubules mediating the vesicle delivery system are then stabilized by the formation of a parasite coat, including the tubulogenic protein GRA7, which acts like a garrote that sequesters host endocytic organelles within the vacuolar space. On one hand, these data define a new process allowing the parasite intimate and concentrated access to a diverse range of low molecular weight components produced by the endo-lysosomal system. More generally, they identify a novel mechanism for unidirectional transport and sequestration of host organelles. On the other hand, these results refute the previous dogma asserting complete seclusion of the *Toxoplasma*-containing vacuole from host endo-lysosomal pathways.

[November, 2005-11-09 - 10h30 - ROOM A]

RT08C - Requirement for the Proper Trafficking of an Integral Invasion Protein Complex to Toxoplasma Micronemes

My-Hang Huynh *(Johns Hopkins Bloomberg School of Public Health)*; Jill Harper *(Johns Hopkins Bloomberg School of Public Health)*; Vern Carruthers *(Johns Hopkins Bloomberg School of Public Health)*

Toxoplasma gondii is an obligate intracellular parasite in the phylum Apicomplexa, which also includes other notable pathogens such as *Eimeria, Cryptosporidium*, and *Plasmodium*. *T. gondii* is capable of infecting a broad host range including humans. Infection is generally asymptomatic in healthy individuals, however in HIV/AIDS patients or organ transplant recipients it can cause fatal opportunistic disease (encephalitis or pneumonia) due to acute tissue destruction by the tachyzoite stage. The tachyzoite lytic cycle begins with active invasion of host cells involving the directional release of transmembrane adhesive domains from apical secretory organelles called micronemes (MIC). MIC proteins on the parasite apical surface are thought to then bind to host cell receptors before being translocated by the submembrane actin-myosin motor toward the posterior end, resulting in forward movement and cell penetration. However, recent studies suggest that *Toxoplasma* expresses at least 10 distinct micronemal adhesins and it is unclear whether they have overlapping, or even redundant, roles in parasite movement and cell penetration. A protein complex con-

sisting of the transmembrane adhesin MIC2 and a tightly associated partner, M2AP, is abundantly released from the MICs. MIC2 and M2AP are found in a stable complex soon after their synthesis and remain together while trafficking through the ER/Golgi, through the micronemes, and then onto the parasite surface where they are shed into the surrounding medium following cell invasion. Similar to many proteins in a regulated secretory pathway, *T. gondii* proteins destined for the MICs and rhoptries (another secretory organelle associated with invasion) undergo proteolytic maturation. M2AP is synthesized as a preproprotein, and processed to a mature form lacking the pro domain. Although it has been established that this maturation step occurs beyond the trans-Golgi, the processing compartment has not been pinpointed. Recent data from our laboratory using anti-peptide antibodies to the M2AP pro domain show that proteolytic maturation occurs in a compartment positioned between the Golgi and the mature micronemes. An M2AP propeptide deletion mutant is partially retained in this region along with MIC2, suggesting that the propeptide contains trafficking information. Although cleavage-resistant mutants of proM2AP are correctly targeted to the MICs, they are less efficiently secreted upon activation. Importantly, both of the M2AP propeptide mutants failed to support normal parasite invasion and were partially attenuated in virulence to a degree that is indistinguishable from M2AP knockout parasites. These findings argue for a dual function of the propeptide in microneme trafficking and in secretion. Interestingly, in a conditional mutant where MIC2 expression levels are 95% lower than wild type parasites, the majority of the M2AP is secreted through the dense granules, which is a default secretory pathway in *T. gondii*. This is likely because MIC2 also harbors targeting elements in its cytosolic domain (Di Cristina et al., Mol. Cell Biol. 2000). Additionally, these parasites show severely impaired invasion efficiency, they switch almost exclusively to a non-productive circular gliding motility, and are incapable of establishing an infection in mice when inoculated at a normally lethal dose. These findings serve as a basis for future studies aimed at defining the branch points of protein sorting to the distinct secretory pathways in emphT. gondiiRequirement for the Proper Trafficking of an integral invasion protein complex to Toxoplasma micronemes and at a deeper understanding of the precise roles played by the M2AP propeptide and MIC2 cytosolic motifs in MIC protein trafficking.

[November, 2005-11-09 - 10h30 - ROOM A]

RT08D - Secretory mechanisms in Paramecium

HELMUT PLATTNER *(Dept. Biology, University of Konstanz, Konstanz, Germany)*

A Paramecium cell contains a plethora of membrane-bounded vesicles, each undergoing specific interactions with other membranes. Generally specificity is thought to be guaranteed by a set of SNARE proteins occurring in the respective vesicle and on the target membrane (v- and t-SNAREs) whose interaction is facilitated by the SNARE-specific chaperone, NSF (= N-ethylmaleimide sensitive factor; SNAREs = SNAP receptors; SNAP = soluble NSF attachment proteins). Previously we have established the involvement of NSF in membrane trafficking in Paramecium, including rosette assembly during trichocyst docking (mediating membrane fusion competence). By implication, this indicated the occurrence of SNAREs. We now have established in our laboratory the occurrence of v-SNAREs (synaptobrevin family) and of t-SNAREs (syntaxin family, SNAP-25) as well as their organelle-specific localization and presumable function (R. Kissmehl, C. Schilde, Th. Wassmer, J. Mansfeld, C. Danzer, K. Nuehse, K. Lutter, H. Plattner, unpublished results). We have established the respective gene structures, cloned cDNAs, prepared antibodies (ABs) for Western blots from cell fractions and for immuno-fluorescence and immuno-EM analysis, overexpressed some forms as GFP fusion proteins (also for immuno-EM), and we have performed homology-dependent gene silencing. Moreover, based on information from higher eukaryotes we have established characteristic domain structures eventually complemented by molecular modeling inasmuch as x-ray structures were available from orthologues in other cells. In sum, activities governed by SNAREs in Paramecium range from the different steps of the digestive cycle to endo- and exocytosis etc. In addition we have, over the past years, established Ca2+-signaling during stimulated exocytosis. Essentially we found mobilization of Ca2+ from cortical stores (alveolar sacs, equivalent to inner lamellar complex of apicomplexans), superimposed by a store-operated Ca2+-influx. - Supported by Deutsche Forschungsgemeinschaft.

[November, 2005-11-09 - 10h30 - ROOM B]

RT09A - Mitochondrial genomes of *Eimeria* spp. and other apicomplexan parasites: molecular and phylogenetic features

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The mitochondrial genome (mtDNA) of Apicomplexa has been characterized in *Plasmodium* spp., corresponding to a linear molecule composed by 15-150 tandemly repeated copies of a 6 kb element. In *Theileria*, on the other hand, the genome is comprised by a 7 kb monomeric element. In both organisms, three cytochrome genes were found (*cyt*b, *cox*I and *cox*III), as well as some stretches of rRNA genes, but no tRNA genes were present. The mitochondrial genome of *Toxoplasma gondii* presents many copies distributed on the

nuclear genome, most of them truncated, precluding a clear distinction between the sequences derived from the nucleus and mitochondrion. In case of case of *Crytposporidium*, no mtDNA was found. In *Eimeria tenella*, a coccidian parasite of the domestic fowl, the occurrence of 170-220 kb molecules hybridizing to a mitochondrial probe was reported. Since the mitochondrial genome is maternally inherited (through the macrogametocyte), it represents an interesting target for monitoring cross-infections. In addition, this genome can also be used for phylogenetic and epidemiologic analyses. In order to characterize the mitochondrial genome of *Eimeria* spp., our group has determined the complete mitochondrial sequences of the seven *Eimeria* species that infect the domestic fowl, plus two species of domestic rabbit: *E. coecicola* and *E. flavescens*. The intra-specific variability was also studied by sequencing five distinct strains of *E. tenella* isolated in three different continents. All mtDNA sequences showed a size of circa 6 kb and contained the cytochrome genes reported for other apicomplexan parasites. A multiple sequence alignment, using sequences derived from the nine *Eimeria* species, revealed a cross-similarity of around 90%. A comparison of the mitochondrial genomes of *Eimeria* spp. and other apicomplexan parasites showed a conservation of the gene order and orientation among different species, but not across distinct genera. These "shuffling"events could be related to the replication mechanism of these genomes, primarily based on recombination events and rolling circle activity. Another interesting finding was the high AT content, of circa 65%, with a highly conserved codon usage, strongly biased towards the use of A/T bases at the third position. Regarding intra-specific variability, the five *E. tenella* strains revealed a high conservation of the mtDNA, with only two consecutive thymines being deleted in strains MC (Brazilian) and Wisconsin (North American), when compared to strains H, TA and Wey (isolated in the UK). This high level of conservation has also been observed in mtDNAs of *P. falciparum* strains, and may reflect a very recent common origin of the strains. A phylogenetic reconstruction of the different *Eimeria* species, using both mitochondrial nucleotide sequences and 18S rRNA, showed a good agreement between these markers. Using cytochrome b sequences in a phylogenetic analysis of Apicomplexa, the Coccidia, Piroplasmida and Haemosporida classes were clearly grouped into distinct clades, as should be expected according to their taxonomy. The status of the mtDNA of *T. gondii* is still a matter of investigation. Bioinformatic analyses of *Sarcocystis* and *Neospora* sequences suggest that these parasites may have suffered a similar process of nuclear transfer of mitochondrial sequences, thus representing a common feature of Sarcocystidae protozoa. We now intend to extend this study by confirming if a mitochondrial genome has remained in the organelle of *T. gondii*, despite the nuclear transfer of mtDNA stretches. This aspect will be addressed through co-localization assays using mtDNA probes and a mitochondrion-specific dye (MitoTracker). **Financial support:** FAPESP and CNPq

[November, 2005-11-09 - 10h30 - ROOM B]

RT09B - Gliding through life: the apicomplexan approach to invasion

Timothy Dowse *(Imperial College london)*; Fabienne Plattner *(CMU, University of Geneva)*; Bernardo Foth *(CMU, University of Geneva)*; Dominique Soldati-Favre *(CMU, University of Geneva)*

Toxoplasma gondii is among the most common pathogens of humans and animals. In immunocompetent individuals, this obligate intracellular parasite can establish an asymptomatic life-long chronic infection. In immunocompromized hosts the reactivation of latent T. gondii infection often results in severe life-threatening encephalitis and upon primary infection during pregnancy, toxoplasmosis, can lead to birth defects and death in the congenitally infected fetus. *T. gondii* is a highly adapted intracellular parasite belonging to the phylum of Apicomplexa. A survival strategy, this parasite infects virtually all nucleated cells in its many vertebrate hosts and utilizes a unique form of locomotion termed gliding motility to penetrate tissue barriers and invade host cells during infection. Gliding involves the concerted action of secretory adhesins, molecular motors and factors involved in actin polymerization that are well conserved across the phylum of Apicomplexa. The establishment of an inducible knockout system for *T. gondii* and *Plasmodium falciparum* offers now the opportunity to tackle the function of those essential gene products implicated in the establishment of infection. During invasion, the secretory organelles called micronemes play a critical role by discharging complexes of soluble and transmembrane proteins to the apical pole of the parasite. These proteins bind to host cell receptors and redistribute towards the posterior pole of the parasite via a connection to the parasite actomyosin system resulting in forward movement and cell penetration. During invasion, the complexes of microneme proteins are rapidly released from the parasite surface by the action of microneme protein protease 1 (MPP1), which is affected by 3,4-dichloroisocoumarin (DCI) a serine protease inhibitor, previously shown to block invasion. MPP1 cleaves within the intramembrane domain at a site, which is preserved in a wide range of apicomplexan microneme proteins, suggesting that the protease is present and conserved across the phylum. Rhomboid proteases are polytopic membrane proteins, forming a new family of intramembrane-cleaving serine proteases. Several genes encoding rhomboid-like proteins are conserved in apicomplexan genomes and two members of this family TgROM4 and TgROM5 constitute plausible candidates for MPP1 activity. The parasite motility is powered by myosin A and the possible contribution of other myosin motors in that process is currently under investigation. Ultimately, gliding motion requires intact actin filaments but the mechanism by which apicomplexans control actin dynamics is unknown, A survey of the apicomplexan parasite genomes revealed the presence of genes coding for proteins homologous to known eukaryotic actin polymerization regulating factors including a profilin-like and two forming-like proteins. We are investigating how

these factors may contribute to our understanding of the invasive mechanisms of and *T. gondii* other apicomplexan parasites.

[November, 2005-11-09 - 10h30 - ROOM B]

RT09D - THE TRANSMISSION CYCLE OF *TRYPANOSOMA CRUZI* AND *T. EVANSI* IN THE WILD

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Trypanosoma cruzi and *Trypanosoma evansi* are ancient eukaryotic hemoflagellates that display significantly distinct life strategies. Nevertheless, they exhibit some essential common biological features: both species are eclectic niche colonizers of their numerous mammalian hosts and present complex transmission cycles. In addition, they represent important threats to human welfare, causing human or farm animal disease. In spite of having been described almost 100 years ago, there are still unknown aspects concerning the epidemiology of these two trypanosomatid species. As humans are more and more getting in touch with the wild environment due to deforestation, tourism, migration, exploiting or even wild environment protection activities, the clarification of these knowledge gaps must be filled. The world geographical distribution of *T. evansi* is broader than that of *T. cruzi* that is restriced to the Americas. Nevertheless, while in Brasil *T. cruzi* is found infecting mammals in all biomes, *T. evansi* is prevalent mainly in Pantanal region. *T. evansi* is described as a very homogeneous taxon on the contrary to the extreme heterogeneity of *T. cruzi*. Consequently, the study of the transmission cycle of these parasites must take into account the peculiarities of each biome, its peculiar fauna, climate and human interference pattern. In this sense our studies showed that fragmentation, land use, seasonality and fauna composition are important modulation factors of the transmission cycle of *T. cruzi* and *T. evansi* in the wild. Furthermore, and concerning *T. cruzi*, the genotype TCI demonstrated to be far more dispersed in the wild than TCII that showed a patchy distribution and was only observed in the Atlantic Rain Forest and Caatinga. Moreover, in spite of its more restricted geographical distribution TCII seem to infect a wide wild host range since it was observed infecting marsupials rodents, carnivores and primates. Distinct transmission cycles could be evidenced in a same forest fragment as well as different epidemiological pictures in a same biome. *T. cruzi* transmission in the wild, mainly of TCII, is focal in agreement to Pavlowski's nidality theory. Historically, humans must have gone into the transmission cycle in several occasions and situations. The landmark certainly was the acquisition of sedentary habits and consequent the possibility of domiciliation of *Triatoma infestans*. This was perhaps the most important path towards the emergence of Chagas disease. In addition, the cases of Amazonia and the recent outbreak in Santa Catarina due to oral contamination attest that other mechanisms are also involved. No association of the two main genotypes of the parasite with a given mammal host could be established. *T. evansi* infects almost all extant mammalian species (excepting sheep), including small rodents and marsupials with crepuscular and nocturnal habits. High parasitemias were mainly observed in Capibaras, coatis dogs and horses. Among them, only capybaras maintain high parasitemia without anemia the main clinical outcome of *T. evansi* infection. The complex study of the transmission cycles of these parasites obligatory involves a multidisciplinary approach. Otherwise, misconclusions and improper managementr schedules may be adopted.

[November, 2005-11-09 - 10h30 - ROOM D]

RT10A - Ecto-enzimas in protozoa parasites:looking for a function

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The plasma membrane of cells contains enzymes whose active sites face the external medium rather than cytoplasm. The activities of these enzymes, referred to as ecto-enzymes, can be measured using living cells. Cell membrane ecto-NTPDases are integral membrane glycoproteins that are millimolar divalent cation-dependent, low specificity enzymes that hydrolyze extracellular nucleoside tri- and /or diphosphate. Their physiological role is still unknown. However, several hypothesis have been suggested such as (i) protection from cytolytic effects of extracellular ATP, (ii) regulation of ecto-kinase substrate concentration, (iii) termination of purinergic signaling, involvement in signal transduction and (v) involvement in cellular adhesion. Here we present some evidences showing that in protozoa parasites these enzymes contribute to adenosine generation and to adhesion to host cells.

[November, 2005-11-09 - 10h30 - ROOM D]

RT10B - *Trypanosoma cruzi*: a relationship between metabolism and the intracellular cycle.

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L-proline and D- glucose are the main energy sources in trypanosomatids. In the present work, we studied intracellular proline concentration, proline transport, glucose transports and proposed a relationship between this metabolites and the differentiation of the intracellular stages in *Trypanosoma cruzi.* Taking advantage of a mammalian cell-line auxotrophic for L-proline, we recently demonstrated that L-proline is relevant for the differentiation of the intracellular stages of *T. cruzi.* In fact, in the absence of L-proline, the differentiation cycle of *T. cruzi* is arrested, with most of parasites remaining as intracellular epimastigotes (IE). Also, trypomastigote (TRY) bursting varies with the concentration of L-proline added to the extracellular medium. In order to understand the phenomena, and based on the fact that in several organisms the presence of certain metabolites are sensed by their transporters, we compared the glucose and the L-proline transport. First we characterized the L-proline transport, establishing the existence of two active systems: one of them powered by ATP and the other by H^+ gradient. The IE displayed the highest proline transport activity when compared to the other stages (30 and 13 times higher then the activity displayed by TRY and amastigotes (AMA), respectively). The intracellular proline concentration was determined as 6.6 mM for AMA, 2.7 mM for TRY and 0.7 mM for IE. The transport of glucose is higher in TRY, being 3.5 times the activity observed for IE and no glucose transport was detected in AMA. In conclusion, we showed that L-proline is essential for the differentiation of the intracellular epimastigotes to trypomastigotes. In conclusion, our results suggest that: 1. D-Glc is preferentially consumed by TRY; 2. L-proline is preferentially consumed by AMA from its intracellular pools during replication and differentiation to IE; 3. L-Proline is preferentially consumed from the environment (host cell cytoplasm) during the differentiation from IE to TRY. Supported by: FAPESP and CNPq E-mail: asilber@iq.usp.br

[November, 2005-11-09 - 10h30 - ROOM D]

RT10C - New targets for antimalarial drugs.

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Development of new drugs is one of the strategies for malaria control. The biosynthesis of several isoprenoids was recently described in *Plasmodium falciparum* and some intermediates and final products biosynthesized by this pathway in mammals differ from those biosynthetized in *P. falciparum.* Herein we report the first isolation and biochemical characterization of most downstream intermediates of the MEP pathway in the three-intraerythrocytic stages of *P. falciparum.* These include 1-deoxy-D-xylulose-5-phosphate, (DOXP) 2-C-methyl-D-erythritol-4-phosphate, 4-(cytidine-5-diphospho)-2-C-methyl-D-erythritol, 4-(cytidine-5-diphospho)-2-C-methyl-D-erythritol-2-phosphate, and 2-C-methyl-D-erythritol-2,4-cyclodiphosphate. We could also demonstrate an active biosynthesis of pyridoxine (vitamin B_6) in parasites metabolically labelled with [1-^{14}C]1-deoxy-D-xylulose-5-phosphate. We have also investigated the effect of fosmidomycin, an antibiotic that inhibit DOXP reductoisomerase, on the biosynthesis of each intermediate of this pathway and isoprenoid biosynthesis (dolichols and ubiquinones). To avoid resistance or the rapid emergence of resistance, combinations with antimalarial inhibitors operating at different points in the same metabolic pathway are supposed to be helpful since synergistic drug action is expected. These facts prompted us to evaluate various terpenes, which share a similar chemical structure with the intermediates of the isoprenoid pathway, as potential antimalarial drugs for the combined use with fosmidomycin. Farnesol, nerolidol and linalool showed stronger inhibitory activity on the biosynthesis of the isoprenic side chain of the benzoquinone ring of ubiquinones in the schizont stage. Treatment of schizont stages with S-farnesylthiosalicylic acid and posterior analysis of isoprenylated proteins led to a decrease in the intensity of the band corresponding to the p21^{ras} protein. The inhibitory effect of terpenes and S-farnesylthiosalicylic acid on the biosynthesis of both dolichol and the isoprenic side chain of ubiquinones and the isoprenylation of proteins in the intraerythrocytic stages of *P. falciparum* appears to be specific, because overall protein biosynthesis was not affected. Significant differences occur in the length of the isoprenic side chains of ubiquinone between different organisms, suggesting different enzymes involved in the synthesis of these side chains. While in *P. falciparum* the isoprenic side chains of ubiquinone contain 7-9 isoprenic units 10 unit-side chains are found in humans. Searching for the *P. falciparum* enzyme responsible for the biosynthesis of isoprenic side chain attached to benzoquinone ring of ubiquinones, we cloned and expressed a putative polyprenyl synthase. Polyclonal antibodies raised against the corresponding recombinant protein confirmed the presence of the native protein in trophozoite and schizont stages of *P. falciparum.* The recombinant protein as well as *P. falciparum* extracts showed an octaprenyl synthase activity with the formation of a polyisoprenoid with eight isoprenic units as detected by RP-HPLC, RP-TLC and confirmed by electrospray ionization mass and tandem mass spectrometry analysis. Both the recombinant and the native version of the enzyme have similar Michaelis constants with the substrates IPP and FPP. The recombinant enzyme could be competitively inhibited in the presence of the terpene nerolidol. This is the first report that directly demonstrates

an octaprenyl pyrophosphate synthase activity in parasitic protozoa. Given the rather low similarity of the *P. falciparum* enzyme to its human counterpart decaprenyl pyrophosphate synthase, we suggest that the identified enzyme and its recombinant version could be exploited in the screening of novel drugs. Supported by FAPESP, CNPq, PRONEX and UNDP/World Bank/WHO.

[November, 2005-11-09 - 14h00 - ROOM A]

RT11A - Structural features in biomolecules of *Trypanosoma cruzi* as good targets for chemotherapy.

de Lederkremer, R.M. *(Universidad de Buenos Aires)*

A rational approach to drug discovery relies on basic exploratory biology and biochemistry to identify molecular targets. Focus to structures xenobiotic in the human host would lead to better drugs. In *Trypanosoma cruzi*, we unraveled some of these unique structures. The lipopeptidophosphoglycan (LPPG) (Lederkremer et al., 1976) later defined as glycoinositolphospholipid (GIPL) is the most abundant cell-surface molecule on the epimastigote and metacyclic trypomastigote forms. In this biomolecule three structural features can be pointed out as absent in mammals: the galactofuranose (Gal*f*), the aminoethylphosphonic acid (AEP) and the inositolphosphoceramide (IPC) moiety. Recently, the mystery of the low amount of peptide found in early preparations of LPPG was solved by Ferguson and coworkers (*J. Biol. Chem.* 2005). They could separate and characterize a novel low abundance glycopeptide (NETNES), with a GPI membrane anchor which shares with LPPG the molecular domain of the glycan but lacks Gal*f*. The biosynthesis of Gal*f* has attracted the interest of several groups, mainly because it is a critical component in *Mycobacterium spp.* The two enzymes involved in its incorporation in glycoconjugates: UDP-α-D-Gal*p* mutase, which converts UDP-α-D-Galp into UDP-α-D-Gal*f* and galactofuranosyltransferase have been cloned. We have described the first chemical synthesis of UDP-[6-^{3}H]-α-D-Gal*f*, as a substrate for studies on inhibitors of the Gal*f* transferase, independently of the mutase. On the other hand, Gal*f* together with Gal*p* is also present in the mucins of lineage 1 of *T. cruzi*, but not in strains of lineage 2 which contain only Gal*p*. Being the mucins the acceptors of sialic acid in the trans-sialidase reaction, we chemically synthesized the natural oligosaccharides of the mucins and studied their relative ability to act as substrates for this unique enzyme. Inhibition of trans-sialidase by lactose derivatives will be described. Enzymes involved in the construction and remodeling of lipids of *T. cruzi* are also good targets for drug development. In an early work on the structure of LPPG (Lederkremer et al., BBRC 1978) an inositolphosphoceramide (IPC) was recognized as the lipid moiety. IPC is also an important constituent of the inositolphospholipids in the three stages of *T. cruzi*, unlike mammalian cells which do not synthesize IPC. We proved that Aureobasidin A, a known inhibitor of fungal IPC synthase impaired the differentiation of trypomastigotes to amastigote forms and the synthesis of IPC and GIPLs. Glycoproteins that characterize different stages of *T. cruzi* are anchored by IPC and actively shed to the medium. We proved that IPC is not a substrate for the first steps in the biosynthesis of the GIPLs, and remodeling steps must be working. Thus, IPC synthase and remodelases are also attractive targets in *T. cruzi*. Rosa M. de Lederkremer CIHIDECAR, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Pabellón II, Ciudad Universitaria, 1428 Buenos Aires, Argentina. E-mail: lederk@qo.fcen.uba.ar.

[November, 2005-11-09 - 14h00 - ROOM A]

RT11B - Inositol Phosphorylceramide synthase activity in *Trypanosoma cruzi*

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In protozoan the major protein posttranslation modification is via ethanolamine phosphate (EtNP), between the C-terminal residue of the protein and an oligosaccharide attached to phosphatidylinositol, known as glycosylphosphatidylinositol (GPI) anchor. In some protozoa parasites, such as *T. cruzi* it is suggested that in the dense glycocalyx the most abundant GPI-containing glycoconjugates are the glycoinositolphospholipid (GILP), being the ceramide the lipid portion. Similar to *T. cruzi*, yeasts and fungi are capable to synthesize inositolphosphorylceramide (IPC) and the inositol phosphorylceramide synthase is an enzyme essential for fungal viability. To understand the importance and mechanism of IPC synthesis in *T. cruzi*, we investigated the effects of rustimicin and aureobasidin A (two potent antifungal compounds) on the proliferation of different life-cycle stages of this parasite. The compounds did not interfere with the axenic growth of epimastigotes, nevertheless, aureobasidin A decresead the released of trypomastigotes from infected murine macrophages and the number of amastigotes in a dose-dependent manner. We have demonstrated that *T. cruzi* expresses an IPC synthase activity, detected in microsomal preparations from all life-cycle stages, that transfers inositol phosphate from phosphatidylinositol to the C-1 hydroxy group of C6-NBD-cer (6-[N-(7-nitro-2-1,3-benzoxadiazol-4-yl)-amino]-hexanoyl ceramide) to form inositol phosphoryl-C6-NBD-cer, which was purified and characterized by its chromatographic behaviour on TLC and HPLC, sensitivity to phosphatidyl-inositol-specific phospholipase C and resistence to mild alkaline hydrolysis. Unlike yeast IPC synthase, the *T. cruzi* enzyme is stimulated by Triton X-100 but not by bivalent cations, CHAPS or fatty acid-free BSA, and it is not inhibited by rustamicin or aureobasidin A, or the two in combination. These results suggested that *T. cruzi* synthesizes its own IPC, by a mechanism that is not affected by antifungal compounds. Supported by CNPq, CAPES, FAPERJ, IFS

[November, 2005-11-09 - 14h00 - ROOM A]

RT11C - Proteomic and Immunological Analysis of Trypanosoma cruzi Shed Vesicles Involved in Host Immunomodulation and Cell Invasion

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Trypanosoma cruzi cell-derived trypomastigotes shed vesicles (Goncalves et al., Exp Parasitol 72:43,1991) that are rich in Tc-85, a GPI-anchored glycoprotein member of the gp85/trans-sialidase (TS) superfamily involved in host cell adhesion and invasion (Magdesian et al., J Biol Chem 276:19382,2001). Here we have fractionated these vesicles in three major populations (P1, P2, and P3) by gel-filtration, followed by affinity chromatography using anti-alpha-galactosyl antibodies from chronic Chagasic patients (Ch anti-Gal). P2 vesicles were shown to be highly glycosylated and able to bind strongly to Ch anti-Gal and anti-Tc85 H1A10 mAb. We have found that these alpha-Gal-containing vesicles (Tc-alphaGalVes) strongly induced proinflammatory cytokines (IL-12 and TNF-alpha) and nitric oxide (NO) in C3H/HeJ and C57BL/6 macrophages, as well as activated TLR2-transfected, but not TLR4-transfected CHO/CD14 cells. These results were substantiated by the finding that macrophages from TLR2-KO mice were unable to produce proinflammatory cytokines and NO upon prior exposure to Tc-alphaGalVes. Surprisingly, we observed that Tc-alphaGalVes dramatically enhanced the trypomastigote ability to invade and multiply inside J774.16 macrophages and TLR2-transfected CHO/CD14 cells. This phenomenon was completely abolished in TLR4-transfected or non-transfected CHO/CD14 cells. To determine their basic protein composition, Tc-alphaGalVes were subjected to proteomic analysis. For this, we used three different protease digestion strategies aiming to increase the number of released peptides. In addition, to increase protein coverage, peptides were fractionated by strong cation-exchange chromatography followed by LC-MS/MS. We reliably identified 126 proteins, which included several members of the gp85/TS superfamily, such as active and inactive TS, and Tc-85 glycoproteins. We also found several polypeptides frequently present in mammalian cell exosomes, and others related to exosomal/vacuolar formation. Taken together our data suggest that Tc-alphaGalVes represent a novel parasite virulence mechanism, which exploits the TLR2 pathway activation to invade host cells. **Supported by BBRC/UTEP/Biology(Grant#5G12RR008124), FAPESP, FAPERJ, FAPEMIG, FIOCRUZ, CNPq, and The Wellcome Trust**

[November, 2005-11-09 - 14h00 - ROOM A]

RT11D - Possible roles of glycosyl-phosphatidylinositols in infections with *Toxoplasma gondii*

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Toxoplasma gondii is a ubiquitous parasite that infects nearly all warm-blooded animals. In healthy individuals *Toxoplasma* infection usually does not lead to clinical symptoms, due to the control of tachyzoites (invasive forms) by the host immune system resulting in their conversion to bradyzoites (latent forms). Developmental switching in *T. gondii*, from the virulent tachyzoite to the relatively quiescent bradyzoite stage, is responsible for disease propagation and reactivation after alteration of the immune status of the carrier. The redifferentiation event is characterized by an over expression of a tachyzoite specific set of glycosylphosphatidylinositol (GPI)-anchored surface antigens and free GPIs. Surface proteins from *T. gondii* grown in normal African green monkey kidney epithelial cells (Vero cell line) have two related GPIs containing a *N*-acetylgalactosamine (GalNAc) residue bound to the first mannose of the conserved three-mannosyl core glycan. One of these two forms contains an additional terminal glucose (Glc) linked to the GalNAc residue. Early IgM immune response, after primary infection with *T. gondii* GPIs, is structure related as sera from infected humans react only with free GPIs containing the Glc residue. The TLC spectrum of free GPIs extracted from *T. gondii* grown in human fibroblasts is characterized by the lack of the GPI-precursor with an unsubstituted GalNAc. GPIs containing terminal glucose bound to GalNAc are found exclusively as free GPIs as they are not transferred to protein in the human cells. Thus, it seems that only GPIs, which are not bound to proteins, give rise to IgM antibodies. Using antibodies raised against free GPIs (glucose-containing) we showed that they are located at the cell surface lipid microdomains that may be involved in the pathology of toxoplasmosis. Acute infection with *T. gondii* results in the production of high levels of pro-inflammatory cytokines, such as IL-12 and TNFα. In

turn, these cytokines initiate the production of IFN-γ and various effector mechanisms that are responsible for the control of parasite growth and pathology during infection with *T. gondii.* However, little is known about the mechanism by which *T. gondii* activates cells from innate immunity and initiates this early synthesis of pro-inflammatory cytokines. Toll-like receptors (TLRs) are the primary means the innate immune system has for recognizing and rapidly responding to the presence of microbes. To date, TLRs have been implicated in the recognition of every known category of microorganisms that causes human diseases. Our recent study suggests that glycosylphosphatidylinositol (GPI) anchors from *T. gondii* tachyzoites have an important role in initiating the synthesis of TNFα by macrophages. We hypothesize that TLRs are central mediators in triggering signalling cascades that will lead to the synthesis of pro-inflammatory cytokines by cells from innate immunity exposed to GPI anchors derived from *T. gondii.*

[November, 2005-11-09 - 14h00 - ROOM B]

RT12A - ANALYSIS OF REGULATORY CD4+CD25+ T CELLS IN CHAGASIC PATIENTS WITH DIFFERENT CLINICAL FORMS OF THE DISEASE

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CD4+CD25high regulatory (Treg) cells, which constitute 1̃0% of peripheral blood CD4+T cells, possess potent regulatory activity of multiple immune functions.Treg cells can influence the immune response to pathogens and the outcome of infectious disease. The expression and role of Treg cells on the development of different clinical forms of Chagas disease has not been demonstrated or established. In this study we analyzed the expression of surface Ag and intracellular cytokine on/in Treg cells to further characterize this regulatory population in chagasic patients. Total leucocytes from the indeterminate (IND, n=14) and cardiac (CARD, n=13) clinical forms of the disease and non-infected individuals (NI, n=11). were cultured for 22 hours in the presence or absence of Trypanosoma cruzi antigens and the phenotypes of CD4+CD25high T cells evaluated by flow cytometry. Our results showed that patients in the IND group have higher levels of CD4+CD25high cells when compared to patients in the CARD and NI groups. The Treg cells from chagasic patients expressed the highest levels of CD45RO, CD62Lhigh and CTLA-4 and lowest levels of CD45RA and CD28 independent of the clinical form when compared with non-infected individuals. CD4+CD25high cells did not express IL-2 intracellular and, in the other hand, these cells expressed higher levels of IL-10, especially in patients from the IND group. Although the analysis of the CD4+CD25high T cells and their role in Chagas disease are important for the understanding of the pathogenesis of this infection, our data demonstrates that this cell population alone is not sufficient to explain the development of the different clinical forms of the disease or determinate their role in human Chagas disease.

[November, 2005-11-09 - 14h00 - ROOM B]

RT12B - Genotypic and phenotypic analysis of co-stimulatory molecules and cytokines in individuals with different clinical forms of Chagas‘ disease: new predictive factors?

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Trypanosoma cruzi is an intracellular pathogen that causes Chagas‘ disease, which remains an important medical problem in Latin America. Up to one-third of infected individuals eventually develop a life-threatening dilated cardiomyopathy, while the majority of infected individuals remain in the indeterminate clinical form. Despite its importance, little is known about which factors govern differential susceptibility to cardiomyopathy development. Since the morbidity in Chagas‘ disease is associated with the host‘s immune response, evaluation of the genetic influences in the outcome of this response may provide new prognostic tools. Previous studies have shown a high frequency of activated T-cells in the peripheral blood (Dutra et al.,94) and cardiac lesions (Reis et al.,93) from chagasic patients. Moreover, cytokine message analysis has shown the presence of both inflammatory and antiinflammatory cytokines in peripheral blood cells of patients with indeterminate and cardiac clinical forms of Chagas‘ disease (Dutra et al.,96), whereas TNF-alpha+ and IFN-gamma+ cells are predominant in cardiac lesions (Higuchi et al.,93; Abel et al.,2001). Some studies have attempted to determine the stimuli responsible for the activation of T-cells in human Chagas‘ disease. Whereas parasite derived antigens are able to stimulate T-cells from chagasic patients *in vitro*, T-cells that can recognize and proliferate to autologous antigens have also been detected in chagasic patients. Induction of efficient cellular immune responses is directly dependent on appropriate activation of antigen presenting cells (APC). T-cell stimulation requires interaction between the TCR and the MHC/peptide complex, as well as adequate co-stimulation provided by the APC. The most important co-stimulatory pathway consists of the interaction between CD28 from T-cells and their counterparts CD80 and CD86 from APC, which leads to lymphocyte proliferation and cytokine production. On the contrary, engagement of CD80 and CD86 by CTLA-4 inhibits T-cell proliferation and the lytic ability. The importance CD28 engagement in T-cell activation has been shown in experimental models of *T. cruzi* infection (Miyahira et al.,2003; Martins et al.,2004). Interest-

ingly, we have demonstrated that chronic chagasic patients present increased levels of CD28- T-cells in their peripheral blood (Dutra et al.1996). Considering the importance of co-stimulatory molecules and cytokines in the generation of the cellular responses in human Chagas' disease, we determined the influence of *in vitro* infection with *T. cruzi* on the expression of such molecules by monocytes and T-cells from patients with the indeterminate or cardiac clinical forms of Chagas' disease. Our results showed that monocytes from indeterminate patients display a 'modulatory profile', as defined by high expression of IL-10 and CD86 and low expression of HLA-DR, whereas monocytes from cardiac patients display an 'activated profile', as defined by high expression of CD80 and TNF-alpha. Moreover, we observed that the expression of CTLA-4 was higher in T-cells from indeterminate patients. We further investigated if the high expression of IL-10 and CTLA-4 by indeterminate patients was associated with the occurrence of functional polymorphisms in the genes encoding for these molecules. Our results showed an association between the presence of high expression alleles of these molecules and the indeterminate form of the disease, suggesting that genetically determined mechanisms are important in the establishment of the mild indeterminate form. These results open new possibilities for prognosis of disease evolution in human Chagas' disease. Financial support: WHO/TDR, CNPq and CAPES.

[November, 2005-11-09 - 14h00 - ROOM B]

RT12C - REGULATION OF LEISHMANIA INFECTION BY INTERACTION OF MACROPHAGES AND NEUTROPHILS

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Apoptosis is a constitutive feature of complex organisms, and can also be induced in the course of inflammatory and immune responses. Neutrophils are among the first cells recruited during inflammation. Neutrophils constitutively die by apoptosis at inflamed sites, and are ingested by macrophages. We investigated how phagocytic clearance of senescent neutrophils interferes with intracellular infection of macrophages by *Leishmania major*. Phagocytosis of dead neutrophils exacerbated *L. major* infection in BALB macrophages through a mechanism dependent on prostaglandin and TGF-beta production. However, phagocytosis of dead B6 neutrophils induced killing of intracellular Leishmania by a mechanism involving TNF-alpha and reactive oxygen species. Leishmanicidal activity was mediated by a soluble factor released from neutrophils. The opposing effects of BALB and B6 neutrophils were confirmed in vivo by experiments of neutrophil depletion and adoptive transfer. A specific inhibitor of Neutrophil Elastase (NE) reverted the leishmanicidal activity of B6 neutrophils, and increased *L. major* infection in vivo. Release of NE is much higher in inflammatory B6, compared to BALB neutrophils. Purified NE induces Leishmania killing by macrophages through a mechanism dependent on Toll-like receptor (TLR)-4 expression on macrophages. Studies with recombinant congenic strains confirmed that macrophage activation by dead neutrophils is polymorphic. These results indicate that phagocytic clearance of dead neutrophils plays an unanticipated regulatory role in Leishmania infection. Financial support: CNPq, FAPERJ, PRONEX, John Simon Guggenheim Foundation and Howard Hughes Medical Institute

[November, 2005-11-09 - 14h00 - ROOM B]

RT12D - Immunoregulation in experimental *Trypanosoma cruzi* infection: new perspective for Chagas disease?

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In Chagas' disease, the establishment of inflammatory processes is crucial for *Trypanosoma cruzi* control in target tissues and the regain of host/parasite equilibrium. However, in about 30% of the patients inflammation becomes progressive, resulting in chronic disease, mainly characterized by myocarditis. Although several hypothesis have been raised to explain the pathogenesis of chagasic myocardiopathy, including the persistence of the parasite, participation of autoimmune processes and imbalanced immune response, the molecular mechanisms underlying the establishment of the inflammatory process leading to parasitism control but also contributing to the maintenance of *T. cruzi*-elicited chronic myocarditis remain unsolved. The comprehension of the pathogenesis of *T. cruzi*-elicited myocarditis is crucial to delineate new rational therapeutic strategies aiming to control non-beneficial inflammation, which leads to heart dysfunction, without hampering the protective immunity involved in parasite growth control. Trying to shed light on these questions, we have been working with murine models that reproduce several aspects of the acute and chronic *T. cruzi*-elicited myocarditis (Talvani et al. 2000, dos Santos et al. 2001, Michailowsky et al, 2004, Marino et al., 2004). Herein, using immunomodulatory strategy we bring evidence that the massive influx of inflammatory cells bearing the CC-chemokine receptor CCR5 into the cardiac tissue is not crucial for cell-mediated anti-*T. cruzi* immunity, however it seems to be critical for pathogenesis of *T. cruzi*-elicited myocarditis. Further, using a TNFR1(p55)-deficient mice, we bring evidence that TNF-alfa participates in recruitment of inflammatory cells, particularly $CD8^+$, to the cardiac tissue during the acute phase of *T. cruzi* infection. Thus, the modulation of TNF-alfa expression became an at-

tractive therapeutic target to be evaluated during *T. cruzi* infection. Our results point to the involvement of TNF-alfa in the pathogenesis of *T. cruzi*-induced myocarditis. Altogether, we bring evidence that CC-chemokine receptors antagonists and TNF-alfa modulators might become attractive therapeutic tools for further evaluation during *T. cruzi* infection. E-mail: lannes@ioc.fiocruz.br

[November, 2005-11-09 - 14h00 - ROOM D]

RT13A - Phytomonas serpens expressing the T. cruzi gene LYT1 is able to multiply in macrophages

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Trypanosoma cruzi invades host cells within an acidic phagolysosomal vacuole that must be disrupted for the parasite to initiate binary division in the cytoplasm. The T. cruzi LYT1 gene encodes a 552-aa protein (mLYT1) with haemolytic activity at pH 5,5 that is expressed mostly by the infective stages. Through alternative trans-splicing, non-infective epimastigotes express a shorter form of LYT1 (kLYT1) that lacks the first 29 amino acids where a putative, computer-predicted type-II anchor signal is encoded. LYT1 null mutants show two apparently unrelated phenotypic characteristic: 1) reduced haemolytic activity at acid pH and reduced virulence and 2) accelerated in vitro stage development. We have found that expression of mLYT1, but not kLYT1, by the null mutant restores haemolytic activity and virulence. mLYT1 is a membrane-anchored protein whose haemolytic activity is required for the parasite to escape from the phagolysosomic vacuole into the cytoplasm. The type-II anchoring signal is recognized in other tripanosomatids: both Leishmania tropica, a human pathogen, and Phytomonas serpens, a phytoflagellate, express mLYT on their surface after transformation with the appropriate constructions. Further, when P. serpens was transfected with a construct engineered to express the first 21 amino acids from mLYT1 fused to eGFP, the chimera was located on the parasite cell surface indicating that the amino terminal region of mLYT1 is necessary and sufficient for anchoring LYT1 to the cell surface. After being engulfed by macrophages mLYT1-expressing, transformed P. serpens are able to free themselves from the vacuole and multiply in the macrophage cytoplasm. On the other hand, expression of the shorter form kLYT1, but not mLYT1, reverts the accelerated development phenotype. In the absence of anchoring signal, kLYT1 localizes in the kinetoflagellar zone, the mitochondrial region where kDNA replication occurs. While the precise role of kLYT1 is presently unknown we hypothesise that it interacts directly or through other protein(s) with mitochondrial DNA, regulating its replication or transcription and thus controlling transition speed between developmental stages. Our results indicate that trans-splicing, operating as a stage-dependent switch point in the control of gene expression, determines the localization of LYT1, resulting in exposure or not to acid pH, which in turn ensues two completely unrelated functions. The hypothesis of alternative folding pathways, under the influence of a compartmentalisation switch, represents an elegant evolutionary solution for the dual functionality of the LYT1, a fundamental protein in the T. cruzi cell cycle.

[November, 2005-11-09 - 14h00 - ROOM D]

RT13B - Ras-related GTPases of *Trypanosoma cruzi*: search for families and functions

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The human parasite *Trypanosoma cruzi* undergoes dramatic morphological changes during its life cycle. In the gut of triatomine bugs dividing flagellate epimastigote forms differentiate to infective metacyclic trypomastigotes in the posterior end of the insect digestive tract. In vertebrate hosts dividing non-motile round forms are found inside tissue cells while circulating non-dividing trypomastigotes are present in the blood. In the eukaryotic lineage small GTPases play pivotal role in the control of cell signaling events involved in main important processes such as differentiation, cell growth and survival. Ras superfamily GTPases are regulatory proteins ubiquitously found in eukaryotic cells. These small GTPases have a molecular mass of 19-30 kDa and comprise the Ras superfamily, which is formed by the families Ras, Rab,Rho, Arf/Sar,Ran,Gem/Kir and RJL. GTPases undergo to a general switch cycle: the GTP-bound active complex stage and the GDP-bound inactive form. The switch from the active to inactive form occurs through GTP hydrolysis. The GTPase switch cycle is modulate by several regulators such as the GDP-exchange factors (GEFs) and the GTPase activating protein (GAP). In general, Ras, Rab and Rho family GTPases undergo post-translational isoprenylation on a C-terminal CaaX motif. Appropriate isoprenylation on the C-terminus of small GTPases is important for correctly targeting and anchoring of these proteins to cellular membranes. Aiming to investigate the role of Ras-related GTPases in the *T. cruzi* cell physiology we have described a Rho family orthologue, named TcRho1, and a novel family of small GTPases RJL. Herein, we describe our findings with TcRho1. TcRho1 is the single member of Rho family identified in trypanosomatids, being expressed in all life cycle forms of *T.cruzi*. In higher eukaryotes Rho GTPases play a critical role in cell movement, phagocytosis, intracellular transport, cell adhesion and maintenance of cell morphology. TcRho1 is a bona-fide GTPase, being able to bind and hydrolyze GTP. Immunolocalization studies revealed that TcRho1 protein is probably associated with the Golgi apparatus. This GTPase presents the C-terminus motif CQLF, which is farnesylated by *T.cruzi* farnesyltransferase. *T. cruzi* cells expressing a TcRho1-mutant (TcRho1-δCaaX), lacking the farnesylation motif exhibited poor levels of metacyclic trypomastigotes as observed in metacyclogenesis assays. In fact, TcRho1-δCaaX cells died synchronously at an earlier point during the metacyclogenesis process. To further investigate the role of TcRho1 we constructed dominant positive mu-

tants TcRho1-G15V and TcRho1-Q76L by site-directed mutagenesis and subcloned in pcDNA3.1 vector. NIH-3T3 fibroblasts were transfected and assayed for possible phenotypic modifications. The NIH-3T3 lineages expressing the dominant positive forms of TcRho1-G15V and Q76L displayed decreased levels of migration compared to the control lineage NIH-3T3 pcDNA3.1. This phenotype may reflect distinct cell-substrate adhesion properties expressed by the mutant cell lines. The data above suggest that TcRho1 is involved in the metacyclogenesis of *T. cruzi* by a process not fully characterized yet. The observations raised in 3T3 fibroblasts with TcRho1 dominant-positive expression indicate that ectopic expression of TcRho1 originates a phenotype associated with cell-substrate adhesion as corroborated by enhanced substrate-adhesion observations. Moreover, fluorescence microscopy of actin filaments stained with phalloidin revealed that fibroblasts expressing TcRho1-G15V and TcRho1-Q76L mutants exhibited numerous and thick filopods. Whether these observations are significant in the context of epimastigote substrate adhesion and further differentiation to trypomastigote remain to be elucidated. It is intriguing that only *T.cruzi* presents a Rho orthologue which may reflect the conservation of specific signaling pathwaysinvolved in the peculiar cell cycle of this parasite. Interestingly, we have identified a putative TcRho1GAP, named TcOCRL, whose sequence predicts a conserved RhoGAP domain and a phosphatidylinositol 4,5-biphosphate(PIP2) 5-phosphatase function. The conservation of this putative TcRhoGAP in *T. cruzi* may reflect the convergence of PIP2 and Rho signaling.

[November, 2005-11-09 - 14h00 - ROOM D]

RT13C - Real-time phagocytosis of Leishmania parasites studied with defocusing microscopy

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Thin transparent objects (phase objects) can be observed in an ordinary light microscope, if the microscope is slightly defocused. Recently, we developed a physical model of a defocused microscope and we found that the contrast of the defocused image is proportional to the amount of defocusing and to the curvature of the phase object. Our model predicts that a phase object plays the role of a small lens that converge or diverge light causing dark or light image contrast. We named this technique defocusing microscopy. Thick phase objects are seen in an ordinary light microscope because some of their parts are always out of focus. We use defocusing microscopy to study motility of macrophages. We observed two types of surface fluctuations: small random fluctuations that permeate the whole cell and large coherent structures (ruffles) that propagate along the cell. Since curvature is one of the most important and energy costing deformation of cell surfaces, our technique can measure a parameter directly related to energetic processes involved in cell motility. With the use of an optical tweezers we can grab a Leishmania parasite and feed a macrophage. In this way we can video-tape and keep track of a single phagocytosis event. We simultaneously obtain, in real-time, phagocytosis time and motility of a macrophage. We observe a clear correlation between motility and phagocytic capacity of macrophages. We will discuss some recent models of cell motility.

[November, 2005-11-09 - 14h00 - ROOM D]

RT13D - Plasmodium falciparum cytoadhesion: Which role for clag9?

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Sequestration of Plasmodium falciparum-infected erythrocytes (IE) is strongly implicated in the pathogenesis of cerebral malaria and pregnancy-associated malaria and is the target of new intervention strategies. Our laboratory investigates the process of cytoadherence molecule trafficking to the surface of IE in parasite lines selected for binding to different endothelial adhesion receptors, such as CD36 and CSA. Previous studies suggested that the clag9 (cytoadherence linked asexual gene 9) gene located on the right arm of chromosome 9 is directly involved in the binding of IE to CD36. Our results show that clag9 is expressed at very late stages in rhoptries of parasites selected for binding to CD36 and CSA and in the parasitophorous vacuole in ring stages, but not at the onset of sequestration in young trophozoites. The analysis of clag9 mutant parasites revealed that parasites express a member of the var gene family at the surface of IE but these IE are unable to adhere to common adhesion receptors (CD36, ICAM-1, CSA, etc.). Our data indicate that clag9 is not itself an adhesion molecule but modulates (by a yet unknown process) the correct assembly of the adhesive complex at the surface of IE.