SPC-1 – **Opening Conference – Samuel Pessoa Award**
Control and Pathology in *Leishmania BRAiensis* Infection: Lessons from Corte de Pedra

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A Southeastern region of the state of Bahia, BRA, is one of the most important endemic areas of American tegumentary leishmaniasis (ATL). Studies in this place started in the village of Três Braços, but after an outbreak of ATL in Corte de Pedra in 1984 a Reference Center for Diagnosis and Treatment of ATL was built in 1987. Initially, both Leishmania (Viannia) BRAiensis and Leishmania (Mexicana) amazonensis were isolated, but in the last 25 years only *L. BRAiensis* is the causal agent of the disease. Cutaneous leishmaniasis (CL), disseminated leishmaniasis (DL) and mucosal leishmaniasis (ML) are documented in about 90%, 6% and 3% respectively in this area. Moreover, about 22% of household contacts of CL patients are infected with *L. BRAiensis* but do not develop disease and are considered as having subclinical *L. BRAiensis* infection (SC). The control of leishmania is mainly mediated by macrophage (Mφ) activation by IFN-γ but in CL, ML and DL despite a strong Th1 immune response enough parasite persists. In contrast a weak Th1 immune response is observed in SC. Mφ from SC have a low respiratory burst but kill more efficiently leishmania than Mφ from CL. While NO is produced in low levels in human Mφ infected with *L. BRAiensis*, ROS participate in the control of parasite growth. Inhibition of ROS increase parasite load in Mφ from CL, ML and DL, but in subjects with SC infection inhibition of ROS and NO does not change the parasite load. In these individuals leishmania killing occurs rapidly after infection and is associated with a quick maturation of the phagolysosomes. Mφ of subjects with SC are also less susceptible to parasite internalization. CL is characterized by one or more round shape well limited ulcer with raised borders. ML is mainly observed in the nose and appears years after a primary CL but in 17% of the cases the mucosal involvement is concomitant with the cutaneous ulcer. DL is characterized by more than 10 and up to 1000 papular and ulcerated lesions in different areas of the body. Both parasite and host factors participate in the pathogenesis of CL. *L. BRAiensis* in the region of Corte de Pedra is polymorphic and genotypic differences in haplotypes of the chromosome 28 among isolates are associated with severity of the disease and failure to therapy. For instance, isolates of DL are more internalized than isolates of CL in Mφ with both forms of the disease, but isolates of DL proliferate more in DL Mφ than in cells from CL patients. The pathology in ATL is associated with an exaggerated Th1 immune response with high production of IFN-γ, TNF, IL-1β, IL-6 and Granzyme B. Parasites are scarce in histopathologic analysis but parasite load detected by DNA of *L. BRAiensis* or by labelled anti *L. BRAiensis* antibody is high. Both the inflammatory infiltrate and parasite load are associated with severity of the disease and failure to therapy. Activated CD8+ T cells and NK cells are present in the inflammatory infiltrate and together with IL-1β and inflammasome play a key role in the killing of cells and development of the ulcer. A high number of B cells and plasma cells are documented at the lesion site and regulatory B cells produce IL-10 which may contribute to parasite persistence in Mφ. Dermal M1 and M2 Mφ are infected with *L. BRAiensis* but while the majority of infected Mφ have only one or few amastigotes a small number of cells have high parasite load suggesting that Mφ subsets remain infected with a large number of amastigotes. Treatment for CL is a challenge in Corte de Pedra as failure to meglumine antimoniate (MA) is observed in up to 50% of the cases. Additionally, those who respond to therapy take 60 to 90 days to achieve cure. As pathology in ATL is associated with the host inflammatory response, the combination of MA with immunomodulatory drugs increase the cure rate and decrease the healing time of cutaneous ulcers.
Keywords: Cutaneous leishmaniasis;L; BRAiensis;Pathogenesis; Therapy.

CO-01 - **Cyclic AMP signaling and evolution of unusual protein kinase A in kinetoplastids**

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Stage development and host adaptation of vector transmitted parasites require fast and reliable perception of signals from the host environments. Kinetoplastids share with model eukaryotes second messengers and signaling modules like protein kinases, but their connections, activation mechanisms and interaction in signaling pathways differ mostly from the well investigated models. The second messenger cAMP is important for innate immunity subversion of trypanosomes in the mammalian host[1] and for transmission via colonization of the tsetse salivary glands[2]. We begin to understand the role of the large adenylate cyclase family and a new multi-cyclase regulator (CARP3) in these processes. The cAMP effector protein(s) in this pathway are still elusive. Notably, protein kinase A (PKA), the primary mammalian cAMP effector, has lost its regulation by cAMP. Nucleoside ligands adopted this role[3]. The structural basis and evolutionary origin of ligand and activation specificity of PKA orthologues and paralogues has been investigated by crystallography, binding assays and a large number of site directed mutants in vitro and in vivo. The repurposing of PKA for novel ligands and pathways other than cAMP evolved in the Euglenozoa. In *T. brucei*, PKA is activated by two environmental cues that trigger differentiation: low temperature (cold shock) and carbon source availability. In *Leishmania*, another unusual PKA isoform tehered to the subpellicular microtubules is essential for maintenance of the elongated shape of promastigotes. Multiple roles in essential adaptive processes in the parasitic life cycle and the unique ligand specificity thus suggest PKA as an attractive target to therapeutically address parasitic diseases caused by kinetoplastids.

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contact presenting author at boshart@lmu.de **Keywords**: signaling;cyclic AMP;protein kinase A.

CO-02 - **IMMUNE RESPONSES IN NAIVE DOGS EXPOSED TO UNINFECTED *LUTZOMYIA LONGIPALPIS* BITES**

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Canine visceral leishmaniasis (CVL) is a disease caused by the parasite Leishmania infantum and transmitted to both dogs and humans through the bites of infected sandflies. In BRA, the primary vector responsible for transmitting the disease is the sandfly species Lutzomyia longipalpis. During feeding, infected sandflies inject metacyclic promastigote forms of the Leishmania parasite, along with their saliva and other components, into the host's bloodstream. Research has shown that antibodies against sandfly saliva are associated with increased severity of visceral leishmaniasis in naturally infected dogs. At the bite site, different inflammatory responses occur, which are mediated by salivary proteins, exosomes and promastigote secretory gel, as well as the microbiota of the sandfly. All of these compounds modulate the host´s hemostatic, inflammatory and immune responses, directly influencing the establishment of Leishmania infection. The early inflammatory response of naïve dogs locally at the site of the sandfly bite can identify which components are more

likely to influence the successful establishment of Leishmania infection In order to assess early skin immune response to uninfected sandflies bites, six dogs were employed: Three 3mm diameter skin biopsies were collected before the exposure from each dog. Exposure was performed using uninfected sandflies from the IGM colony. Total RNA was extracted and the integrity and quantification of the mRNA was confirmed by automated electrophoresis and the cDNA library was built using the Ion total RNA-seq V2 kit. The majority, approximately 70%, of the 295 DEGs (differentially expressed genes) identified by the LinDA analysis were related to the first time point evaluated, 4 hours after saliva exposure. The 24-hour time point had fewer DEGs, while a larger number were present at the later evaluated time point, 48 hours. About 13% of the DEGs could not be mapped in the enrichment analysis. Thus, this analysis was performed using the remaining 255 DEGs. These genes revealed 28 enriched pathways after exposure to sand fly saliva. The IL-17 signaling pathway was the only pathway related to the 24-hour time point and was also associated with the initial 4-hour time point. Pathways related to leishmaniasis, tuberculosis, phagosomes, and chemokine signaling were enriched only at the later evaluated time point. Out of the 28 enriched pathways, 18 were specifically associated with the initial time point. These pathways were associated with the inflammatory response and some commonly associated with chronic diseases such as Chagas disease, African trypanosomiasis, and rheumatoid arthritis. Cytokine genes related to IL1B, IL6, CXCL8, and CCL2 were shared by different pathways associated with the 4-hour time point and some pathways related to the 48-hour time point. Since the FCGR1A gene is connecting pathways related to the early time point after exposure to sand fly saliva with those related to the later 48-hour time point, it may be playing a role in the response at these two time points. Additionally, antibodies against sandfly saliva can be useful in assessing vector exposure in endemic areas. Recombinant proteins derived from sandfly saliva, such as LJM11 and LJM17, have been employed to detect anti-saliva antibodies in dogs. In an experimental study, six dogs were exposed to Lu. longipalpis sandflies, and their production of anti-LJM11 and anti-LJM17 antibodies was monitored over time. The dogs showed an immediate increase in antibody production after the first exposures, and the antibody titers remained detectable for over a year, with variations observed among individual animals. Upon re-exposure to sandflies, the dogs exhibited a significant rise in antibody titers. This study allows us to better understand of immune responses in dogs, contributing to the design of new prophylactical vaccines, considering the role played by the sand flies in the establishment of infection.
Supported by: PROEP IGM-FIOCRUZ N°01/2020 and Fulbright Junior Member Faculty Award
Keywords:: Sandfly;Saliva;Antibodies; Reservoir.

CO-03 - **Extracellular vesicles from the parasite *Trichomonas vaginalis:* role in cell communication**

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Trichomonas vaginalis is a common sexually transmitted parasite that colonizes the human urogenital tract where it remains extracellular and adheres to epithelial cells. Infections range from asymptomatic to highly inflammatory, depending on the host and the parasite strain. With an estimated annual prevalence of 276 million new cases, mixed infections with different parasite strains are expected. Although it is known that parasites interact with their host to enhance their own survival and transmission, evidence of mixed infections call into question the extent to which unicellular parasites communicate with each other. We recently demonstrated that different T. vaginalis strains can communicate through the formation of cytoneme-like membranous cell connections. We showed that cytonemes formation of an adherent parasite strain (CDC1132) is affected in the presence of a different strain (G3 or B7RC2). Our findings provide evidence that this effect is contact-independent and that extracellular vesicles (EVs) are responsible, at least in part, of the communication among strains. EVs are heterogeneous membrane vesicles released from virtually all cell types that collectively represent a new dimension of intercellular communication. We found that EVs isolated from G3, B7RC2, and CDC1132 strains contain a highly distinct repertoire of proteins, some of them involved in signaling and communication, among other functions. Finally, we showed that parasite

adherence to host cells is affected by communication between strains as binding of adherent *T. vaginalis* CDC1132 strain to prostate cells is significantly higher in the presence of G3 or B7RC2 strains. We also observed that a poorly adherent parasite strain (G3) adheres more strongly to prostate cells in the presence of an adherent strain. The study of signaling, sensing, and cell communication in parasitic organisms will enhance our understanding of the basic biological characteristics of parasites, which may have important consequences in pathogenesis.
Supported by:NIH **Keywords:** parasite;communication;vesicles.

CO-04 - **Exploring host-specificity: Unraveling the relationship between *Leishmania (Viannia)* species and its endosymbiont *Leishmania* RNA Virus 1**

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An important aspect in the epidemiology of Tegumentary Leishmaniasis (TL) in endemic regions of northern South America, as well as certain areas in Central America, is the presence of Leishmania parasites carrying a viral endosymbiont known as Leishmania RNA Virus 1 (LRV1). LRV1 is a double-stranded RNA virus found in various species of Leishmania (Viannia). Some studies have indicated that Leishmania parasites carrying LRV1 are more likely to cause severe symptoms of TL, increasing the risk of progression to the mucosal form of the disease. However, while the association between LRV1 and mucosal manifestation has not been observed in all studies, there is evidence linking LRV1 to therapeutic failure. These inconsistencies may be attributed to differences in the parasite populations circulating in the various regions studied and/or variations in the LRV1 strains infecting these parasites. LRV1 has been detected in cultivated strains of *L. BRAiensis*, *L. guyanensis*, *L. naiffi*, *L. panamensis*, and *L. shawi*, but not in other *Leishmania (Viannia)* species. Although LRV1 has been associated with human infections caused by *L. lainsoni* and *L. peruviana*, cultivated strains of these species have yet to be identified as LRV1 positive. Previous studies employing complete genome sequences of LRV1 and sequences obtained from a phylogenetically informative region of the viral genome from *L. guyanensis* and *L. BRAiensis* have demonstrated evidence of host-specificity in the interaction between *L. (Viannia)* species and LRV1, with LRV1 sequences clustering according to their respective Leishmania species hosts. Host-specificity has also been observed in the analysis of LRV1 from *L. shawi* and *L. naiffi*. LRV1 from L. shawi clustered closely with LRV1 from L. guyanensis, mirroring the observed phylogenetic relationship between these two Leishmania species. All LRV1 sequences from *L. naiffi* clustered together, forming the most divergent group. It is worth noting that phylogenetic analysis suggests *L. naiffi*, along with *L. lainsoni*, as the most divergent species within theViannia subgenus. Given the significance of the Leishmania-LRV1 symbiosis in the epidemiology of cutaneous and mucosal leishmaniasis, it is crucial to obtain a comprehensive understanding of the diversity and spread of this virus within parasite populations.
Keywords:: Leishmania (Viannia);Leishmania RNA Virus 1;host-specificity.

CO-05 - **The ultrastructure of cell division coordination in *Toxoplasma gondii***

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The ability of protozoan parasites to rapidly proliferate within their host is at the core of their mechanisms of pathogenesis. Obligate intracellular parasites of the apicomplexan phylum lyse their host cells and tissues as a consequence of their cell division. Parasites of this phylum resort to flexible cell division modes resulting in variable outputs. *Toxoplasma gondii*, the causative agent of toxoplasmosis, for example, is able to proliferate by means of endodyogeny, endopolygeny and schizogony. These modes of division share mechanistic features such as lack of chromatin

condensation, nuclear fission by semi-closed mitoses and *de novo* daughter cell assembly. However, the underlying mechanisms of this flexibility have only recently started to emerge. The centrosome, one of the microtubule organizing centers in the cell, has long been staged at the center of regulation. Here, we have dissected the contribution of different centrosomal components in *T. gondii*, highlighting their individual contributions orchestrating distinct phases of endodyogeny. Using ultrastructure expansion microscopy, we have analyzed the phenotypes displayed by a number of conditional mutants of centrosomal proteins uncovering their unexpected roles in microtubule nucleation, centriole biogenesis and regulation. Overall, our work proposes a model for the modular organization of centrosomal functions which ultimately underlies cell division flexibility, allowing these parasites to adapt to different niches and proliferate accordingly. Su**pported by:** Pasteur Network
Keywords:: Toxoplasma gondii;Cell Division;UExM.

CO-06 - **Exploring Exosome-Like Vesicles in *Giardia lamblia*: Implications for Parasite Communication, Pathogenicity, and Drug Resistance**

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Extracellular vesicles (EVs) play a vital role in intercellular communication and their potential to promote pathogenesis in parasites has generated significant interest in the medical field. These vesicles involve microvesicles (MVs) and exosomes, which selectively transfer proteins, lipids, mRNA, and microRNA from one cell to another. While MVs are formed by extrusion from the plasma membrane, exosomes are a population of vesicles of endosomal origin that are stored within multivesicular bodies (MVBs) as intraluminal vesicles (ILVs) and are released when MVBs fuse with the plasma membrane (PM). In this sense, whether exosome biogenesis is entirely separate from ESCRT machinery or driven by it is still debated. In our laboratory, we found that the protozoan parasite *Giardia lambli*a, although lacking a classical endo-lysosomal pathway, can produce and release exosome-like vesicles (ElVs) in terms of size, shape, lipid, protein, and small RNAs composition. Our results indicated that ILV formation and ElV release are dependent on the ESCRT-associated AAA+-ATPase Vps4a and ceramide in this parasite. We also observed that the proteomic analysis of ElVs of genetic subtypes (assemblies) A and B, contain parasite-specific as well as EVs-conserved protein. Similar results were observed when we analyzed the RNA cargo. RNA sequencing analysis revealed that the ElVs of each assemblage contained distinct small RNA (sRNA) biotypes, suggesting a preference for specific packaging. These sRNAs were classified into three categories: ribosomal-small RNAs (rsRNAs), messenger-small RNAs (msRNAs), and transfer-small RNAs (tsRNAs), which may play a regulatory role in parasite communication and contribute to host-specificity and pathogenesis. The uptake experiments showed that ElVs were successfully internalized by the parasite trophozoites and that the RNAs were first located below the plasma membrane but then distributed along the cytoplasm. These investigations are part of the central objective of our project, which is to investigate whether there is information transmission between parasites that could define the role of EVs in drug resistance, pathogenicity, and cell survival of *G. lamblia*. **Supported by::** Agencia Nacional para la Promoción de la Ciencia y Tecnología, Argentina, grant number PICT2018-713 and PICT-2021-CAT-II-00073.
Keywords:: Extracellular vesicles ;Intercellular communication;Small RNAs (sRNAs).

CO-07 - **Characterizing the diversity of eukaryotes in the primate gut**

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Eukaryotes are a normal part of the mammalian gut ecosystem. They have historically been studied as parasites but high prevalence in healthy individuals suggest they may be indicators of a healthy gut ecosystem. Diversity in the gut is declining in response to modern lifestyles for both humans and non-human primates in captivity, but the consequences of these changes are not clear. I will present data from human populations and wild non-human primates that broadens our understanding of the diversity and variation of the eukaryome in health and disease. In a large cohort of children from Madagascar and Central African Republic (Afribiota), we find that the eukaryome is poorly correlated with clinical variables including chronic undernutrition, anemia, intestinal inflammation, and age. Interestingly, we find lower levels of the common gut protist Blastocystis in stunted children compared to non-stunted controls, mirroring findings that Blastocystis is less common in individuals with inflammatory or gastrointestinal disease compared to healthy individuals in other parts of the world. Across studies, we find high inter-subject variability, frequent co-occurrence of eukaryotes within an individual, and weak correlation with host factors. These patterns often contrast with those observed in the bacterial microbiome. Throughout, we use phylogenetic placement to refine taxonomic identification of gut eukaryotes and find only a handful eukaryotes that are likely residents of the gastrointestinal tract, while much of the signal from 18S rRNA gene metabarcoding data comes from transient organisms and from dietary and environmental sources. Our results highlight the importance of studying populations across the world to uncover common features of the eukaryome in health.

CO-08 - **RNA editing in non-model kinetoplastids**

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The kinetoplastids are unicellular flagellates that derive their name from the ‘kinetoplast’, a region within their single mitochondrion harboring its organellar genome of high DNA content. Some protein products of this mitochondrial genome are encoded as cryptogenes; their transcripts require editing to generate an open reading frame. This happens through RNA editing, whereby small regulatory guide (g)RNAs direct the proper insertion and deletion of one or more uridines at each editing site within specific transcript regions. An accurate perspective of the kDNA expansion and evolution of their editing across kinetoplastids has been difficult to achieve. Here, we resolved the kDNA structure and editing patterns in the early-branching kinetoplastid *Trypanoplasma borreli* and compare them with those of the well-studied trypanosomatids. We find that its kDNA consists of circular molecules of about 42 kb that harbor the rRNA and protein-coding genes, and 17 different (likely, linear) contigs of approximately 70 kb carrying an average of 23 putative gRNA loci per contig. Our analysis uncovered a putative gRNA population with unique length and sequence parameters that is massive relative to the editing needs of this parasite. In addition, we analyzed RNA editing in another non-model species, *Blastocrithidia nonstop,* a trypanosomatid species with all 3 stop codons reassigned to encode amino acids, and revealed that (with some background noise) it is limited to just 2 cryptogenes, *RPS12* and *COIII.*
We conclude that the organization of kDNA across known kinetoplastids represents variations on partitioned coding and repetitive regions of circular molecules encoding mRNAs and rRNAs, while gRNA loci are positioned on a highly unstable population of molecules that differ in relative abundance across strains. Likewise, while all kinetoplastids possess conserved machinery performing RNA editing of the uridine insertion/deletion type, its output parameters are species-specific.
Supported by:: GA CR 22-01026S **Keywords::** Trypanosomatidae;RNA editing;non-model species.

CO-9 - **Update of Malaria-associated Acute Respiratory Distress Syndrome**

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Malaria, still present in 84 malaria-endemic countries, caused 619,000 deaths in 2021. Different murine models are used to study severe malaria, such as cerebral malaria, hepatic and renal insufficiencies, placental malaria, severe anemia, and acute respiratory distress syndrome (ARDS), leading to the death of infected patients. This syndrome is characterized by acute inflammation, alveolar endothelium and pulmonary parenchyma injuries, dysfunction and increased permeability of the pulmonary alveolar-capillary barrier, and, consequently, edema formation. Thus, the lecture will address different aspects of the pathogenesis and the possibility of syndrome biomarkers. We observed that the integrity of the endothelial barrier is modified by the presence of erythrocytes parasitized with P. berghei ANKA, and there are changes in the actin, myosin, and microtubule cytoskeleton. In addition, results regarding the lungs and serum of mice with malaria-associated ARDS by quantitative proteomic analysis will address. In the lungs, 32 proteins were found upregulated only in mice that developed ARDS on days 7 and 9 dpi. Proteins involved in several processes, such as complement and coagulation cascade activation, neutrophil migration, extracellular matrix organization, and regulation of actin cytoskeleton, were found to be regulated, confirming preliminary results from our group. We characterize the expression of serum proteins in malaria-associated ARDS, especially acute phase proteins, to potentially identify early disease biomarkers. The integration of lung and serum proteomes data has been evaluating severe malaria with associated pulmonary complications that could spread a novel outlook on malaria-associated ARDS. **Supported by:**FAPESP 2020/03163-1 **Keywords:** malaria;acute respiratory distress syndrome;murine model.

CC-01 - **What we have learned about *Trypanosoma cruzi* endocytic pathway**

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Our group has been directly studying the endocytic pathway of *Trypanosoma cruzi* for 45 years. In this long way, we used the best tools we had to describe the macromolecule entry portals and the path they take to the compartment where they are stored, which we had called reservosomes. As the lysosomes are the last organelles of the endocytic pathway in all eukaryotic cells studied until now, we have also been looking for *T. cruzi* lysosomes, until we gave up and admitted that lysosomes are the reservosomes themselves. But not all of them... *T. cruzi* lysosome-like organelles are not homogenous, concerning their size, internal pH and enzymatic content, as mammal cell lysosomes are also not. In the last 10 years we have also contributed significantly to the structural knowledge of the cytostome-cytopharynx complex and its dynamics (and what dynamics!) in the epimastigote cell cycle and in the whole life cycle of *T. cruzi*. After many observations and quantifications, we can support that the cytostome is the main entry site for macromolecules in the proliferative forms, epimastigotes and amastigotes. But what about the flagellar pocket? Other trypanosomatids lacking the cytostome, such as *T. brucei* and *Leishmania sp* uptake molecules for nutrition and defense through the flagellar pocket, but in *T. cruzi*, records of a tracer at the flagellar pocket are extremely rare. On the other hand, we know very little about *T. cruzi* secretion pathways. Would the pocket membrane domain be a priority site of secretion? Or could macromolecules find their way out going upstream along the cytopharynx, as may be suggested by the acidic pH of its lumen and the proximity to the Golgi complex?
We urgently need secretory tracers and molecular markers to the *T. cruzi* endocytic compartments! Or another 45 years of observations... **Keywords**: Endocytosis;Cytostome;Flagellar pocket.