SPC1 - **TRYPANOSOMA CRUZI, A FLAGELLATED PARASITE SEEKING SHELTER FROM THE STORM: MULTIFACETED ROLES OF BRADYKININ-INDUCED PROTEOLYTIC CASCADES**

SCHARFSTEIN, J.*[1]

1.UFRJ-IBCCF, RJ, Brazil.

Traditional viewpoints on the mechanisms by which opportunistic pathogens escape from innate immunity have highlighted the importance of strategies that either down-modulate or suppress anti-microbial immunity. For decades, the Complement system was considered the paradigm of a proinflammatory serine protease cascade that links inflammation to immunity. However, there is now awareness that role of clotting in mammalian immunity was overlooked for the simple reason that coagulation research was dominantly focused on hemostasis/thrombosis. Although the invertebrate and mammalian serine protease cascades that mediate clotting originated through convergent evolution, formation of clots by limited proteolysis is a conserved mechanistic feature of insect immunity. In an important twist in this field, the notion that coagulation provides the first barrier of host defense against bacteria in mammals was supported by mice studies (fibrinogen deficient strains) showing that bacterial clearance by phagocytes required microbial retention in fibrin scaffolds formed by cross-talk between the extrinsic and intrinsic (contact-dependent) pathways of coagulation. Also referred as the Kallikrein-kinin system (KKS), the plasma contact system is composed of (i) Factor XII (FXII) and prekallikrein (PK) (serine protease zymogens) (ii) high molecular weight kininogen (HK), a non-enzymatic contact co-factor that also serves as the precursor of bradykinin (BK) and (iii) C1 inhibitor (serpin inhibitor of PKa and FXIIa). It is well-established that the contact system/KKS can be vigorously triggered by negatively charged platforms exposed in microbial surfaces or by endogenous polymers such as polyphosphates (externalized by activated platelets/mast ells), heparin (mast cells) or DNA associated to neutrophil extracellular traps (NETs). After multiple cycles of reciprocal activation between FXII/PK, FXIIa triggers thrombin-dependent fibrin formation via the intrinsic (FXI/FIX) pathway whereas PKa liberates the short-lived bradykinin (BK) nonapeptide from HK. Tightly regulated by metallopeptidases, such as ACE, the short-lived BK induces vasodilatation and increases microvascular permeability via activation of endothelial B2R, a constitutively expressed GPCR.

Although the promise of untangling the role of the KKS in the pathogenesis of Chagas disease is still distant, studies of the mechanisms integrating inflammation to immunity suggested that kinins play a dichotomous role in the unstable host/parasite relationship. Acting on the behalf of the immunocompetent host, kinins liberated in splenic sinusoids or peripheral tissues upregulate/sustain type-1 effector T cell development via B2R-dependent activation of antigen-bearing dendritic cells. A more complex picture emerged when we studied the impact of innate recognition of trypomastigotes in the inflamed peripheral tissues. Acting in concert with innate immune cells, the proteolytically released kinins and C5a anaphylatoxin propagate infection-associated inflammation via iterative cycles of mast cell degranulation, BK release and activation of plasma-borne contact factors. Taking advantage of the elevated levels of BK and endothelins released in the edematous tissues, T. cruzi trypomastigotes seek “shelter from the inflammatory storm” by invading heart cells via cross-talk between G-protein coupled bradykinin and endothelin receptors. Of potential interest to vascular theories of pathogenesis in Chagas disease, intravital microscopy studies (hamster cheek pouch model of infection) revealed that tissue parasitism triggers inflammatory neovascularization via the mast cell/chymase pathway. Work in progress may clarify whether angiogenic responses induced by intracellular parasites might facilitate the systemic dissemination of trypomastigotes, reminiscent of mechanisms supporting tumor metastasis.

Supported by:CNPQ, CAPES, FAPERJ and INBEB-INCT **Keywords:** Angiogenesis, bradykinin; chagas disease, contact system; immunity, mast cells

CO1 - **KINETOPLASTID CELL BIOLOGY BEYOND THE GENOME: UNDERSTANDING THE LINKS BETWEEN PARASITE STRUCTURE, FUNCTION AND PATHOGENICITY.**

GULL, K.*[1]
1.UNIVERSITY OF OXFORD, United Kingdom.

We have had decent genomic information on the major kinetoplastid parasites for over a decade. That information, coupled with innovations in forward and reverse genetic toolkits and intelligent screens has led to more a global understanding of the molecular cytology of these parasites. Comparative studies between free-living kinetoplastids, T. brucei, T. cruzi and Leishmania now allows reflections on the potential of the intrinsic cell biology in their evolutionary steps to parasitism. After an overview of these issues and some future-gazing on areas of importance and ignorance I will illustrate these themes with reference to work in my laboratory and that of collaborators.

Specifically, I will focus on
• control of flagellum motility and functions intrinsic to achievement of life cycle
• cell surface topography, in particular localised positioning of cytoskeletal and cell surface molecules
• design of the flagellar pocket architecture and host / vector interactions within the life cycle
• cell adhesion – both between parts of the cell surface and between host and pathogen
• intrinsic asymmetries influencing cell type transitions and cell divisions.

Supported by: Wellcome Trust **Keywords:** Trypanosome; cell biology; leishmania

CO2 - **TARGETING CD4+ T CELLS FOR VACCINATION AGAINST INTRA-PHAGOSOMAL PATHOGENS: INSIGHT FROM THE *LEISHMANIA* MODEL.**

PETERS, N.*[1]
1.UNIVERSITY OF CALGARY, Canada.

Leishmania is a vector-transmitted parasite that establishes intracellular chronic infection in mammalian phagocytic cells and is the causative agent of the neglected tropical disease leishmaniasis. Disease varies from localized cutaneous lesions to disseminated visceral infection. Despite the development of protective adaptive immunity following recovery from a single primary cutaneous infection with *Leishmania major,* a vaccine that emulates this protective response remains elusive. Comparison of naturally acquired immunity versus vaccine-induced immunity in animal models has emphasized the importance of an early immune response in order to prevent disease following infected sand fly challenge. This early immune response is largely mediated by circulating $Ly6C^+CD4^+$ T effector (T_{EFF}) cells that are maintained by chronic primary infection and are rapidly recruited to dermal sites of secondary challenge. These cells have also been shown to mediate early protective immunity in the liver and spleen employing a dermal needle inoculation model of visceral infection with L. infantum. In order to determine why this early response appears to be critical for protection we have investigated Leishmania-phagocyte-T cell interactions in the skin at early time points post-challenge. We found that the majority of *L. major* parasites transition from $Ly6G^+$ neutrophils to $CCR2^+CX3CR1^+Ly6C^+$ inflammatory monocytes between 10 and 48 hours post-infection. Infected monocytes played a central role in parasite expansion at sites of primary infection but were rapidly activated to control parasite growth at sites of secondary infection. Monocyte-mediated parasite control at secondary sites was IFN-γ-dependent and associated with enhanced MHC II expression whereas infected monocytes at primary sites had decreased MHC II expression versus uninfected cells from the same dermal environment. In-vitro, monocytes are more permissive for *L.m.* expansion versus bone marrow derived macrophages (BMDM) but are also more efficient at killing parasites following co-culture with $Ly6C^+CD4^+$ T_{EFF} cells from chronically infected mice. In-vivo assessment of the timing of $Ly6C^+CD4^+$ T cell mediated effector function employing adoptive transfer,

short-term IFN-γ neutralization, or blockade of T cell migration into the skin formally demonstrated that effector mechanisms that provide protection when present at the time of challenge provide no protection if delayed until 4 days post challenge, despite the fact that *Leishmania* does not divide during this early time period. These findings suggest an early window exists during which T cell immunity must act in order to prevent the establishment of the parasitic niche. Of interest, comparison of the kinetic of effector responses mediated by $CD44^+CD62L^+$ T central memory (T_{CM}) versus $CD44^+CD62L^-Ly6C^+$ T_{EFF} cells in an adoptive transfer model revealed that $Ly6C^+$ T_{EFF} cells mediate rapid IFN-γ production at the site of challenge. In contrast, T_{CM} cells mediate delayed effector function and do not provide the early effector response that correlates with protection against infected sand fly challenge. However, adoptive transfer of T_{CM} cells into chronically infected-infection matched recipients revealed that T_{CM} cells generate $Ly6C^+$ T_{EFF} cells upon exposure to chronic infection. These findings suggest the role of $CD4^+$ T_{CM} during *Leishmania* infection is not to generate effector cells following challenge, but rather to provide a pre-selected pool of Th1 cells that generate circulating $Ly6C^+$ T_{EFF} cells upon exposure to chronic antigen prior to secondary challenge. In this way, T_{CM} cells maintain the T_{EFF} population required to protect against secondary infected sand fly challenge. These findings have improved our understanding of the immune-biology of the *Leishmania*-host interaction and provide critical correlates of protective immunity that can be employed to better inform vaccine design. **Supported by:**Canadian Institutes of Health Research **Keywords:** Leishmania; cd4 t cells; monocytes

CO3 - **AMINO ACIDS AS A TOOLBOX FOR THE DANGEROUS LIFESTYLE OF *TRYPANOSOMA CRUZI***

SILBER, A.M.[*1]
1.ICB-USP, SP, Brazil.

Taken together, amino acids have a broad spectrum of physicochemical characteristics that make of them natural candidates to buffer the different intracellular environments in terms of pH, redox state and osmolarity. Additionally, their participation in the bioenergetics of different cells is well documented. Therefore, it is reasonable to propose that their uptake and metabolism contribute to regulate cellular processes that are sensitive to these environmental conditions and to the energy status of the cell. *Trypanosoma cruzi*, the causative agent for Chagas disease, has a complex life cycle involving two different types of hosts: reduviid insects and mammals. Inside each host, the parasite faces different environments: the different parts of the digestive tube in the insect host, and the bloodstream, and the cytoplasm of the cells in different tissues inside the mammalian hosts. In each environment the parasite must survive to different stress conditions, mainly redox and nutritional stress. It has been shown by our group and others that amino acids such as proline and its metabolic intermediate P5C play different roles in the cell bioenergetics, differentiation processes, and survival to redox and severe nutritional stresses, and contributes to the metabolic flexibility of *T. cruzi*. Additionally, other amino acids such as histidine, glutamine, and alanine, which are metabolically related to proline, are able to participate in the interplay between redox and bioenergetics metabolism in this parasite. Summarizing, the multi-functionality of amino acids and their intermediate metabolites is critical for *T. cruzi* survival in the challenging environments this parasite transits during its life cycle.

Supported by:FAPESP **Keywords:** Amino acids metabolism; trypanosoma cruzi; stress

CO4 - **KILLING THE MESSENGER:DISRUPTING VECTOR-HOST-PARASITE INTERACTIONS TO CONTROL LEISHMANIASIS**

COSTA, A.G.B.*[1]; VALENZUELA, J.G.[1]; OLIVEIRA, L.F.B.[1]
1.NIAID, United States.

Sand fly saliva is composed by an arsenal of bioactive molecules that not only allow the insect to have a successful blood meal but that also exacerbate Leishmania infection.Upon a bite of an uninfected or infected sand fly, neutrophils rapidly migrate and accumulate at the site of the bite. This observation led us to hypothesize that sand fly saliva possesses a chemo-attractant protein that directly recruits neutrophils.. Chemotaxis driven by sand fly saliva of vectors of visceral (Lutzomyialongipalpis) or cutaneous (Phlebotomus duboscqi) disease was measured by modified Boyden chamber and by the EZ-Taxiscan assays. Blood purified human and dog neutrophils migrated towards saliva of the vectors P. duboscqi and L. longipalpis, in a dose-dependent manner. Moreover, L. longipalpis saliva recruited more human and dog neutrophils than P. duboscqi. Inversely, P. duboscqi recruited bone marrow purified mouse neutrophils in a dose-dependent manner, but L. longipalpis had no impact on mouse neutrophils migration. In search to identify saliva chemo-attractant, we show here that sand fly yellow-related proteins recruit neutrophils in vitro and in vivo. We propose the idea that sand fly salivary yellow proteins act as chemokines during Leishmania infection, recruiting neutrophils to the site of the bite. **Supported by: Keywords:** Sand flies; leishmaniasis; innate immunity

CO5 - **VARIANT GENE TRANSCRIPTION IN *PLASMODIUM FALCIPARUM*: NOVEL INSIGHTS ON *RIF* AND *VAR* GENE TRANSCRIPTION**

WUNDERLICH, G.*[1]; CUBILLOS, E.F.G.[1]; PRATA, I.O.[2]; ARAUJO, R.B.D.[2]; SILVA, T.M.[1]
1.USP-ICB, SP, Brazil; 2.ICB-USP, SP, Brazil.

The human malaria parasite Plasmodium falciparum expresses highly variant proteins on the infected red blood cell surface which partly function as ligands for lymphocyte receptors (RIFINs) or endothelial cell receptors and, in the case PfEMP1, ultimately trigger severe forms of human malaria. We addressed variant gene transcription by producing knock-in strains of *P. falciparum* which targeted either *rif* loci or putative transcription factors which are supposed to control *var* transcription. Although sharing some regulating epigenetic motifs - histone modifications - the expression of the ~180 different RIFINs apparently does not involve strictly limited numbers of active *rif* genes. On the other hand, the transcription of the PfEMP1-encoding *var* gene family is tightly controlled by a dynamic process termed allelic exclusion but how this mode of transcription is orchestrated is still not fully understood. Using conditional knockdown mutant strains, we show that the putative transcription factor AP2-O - PF3D7_1143100 – besides its essential role in oocyst biology - plays also a crucial role in asexual parasite stage forms. Temporary knockdown of AP2-O for two reinvasion cycles led to the activation of a number of *var* loci and the inactivation of a few others. Early gametocyte-stage related genes were also activated but not AP2-G, the master regulator of gametocytogenesis, coincident with the observation that knockdown of AP2-O itself is not causing a switch to gametocyte-like forms and did not influence *in vitro* growth rates. When re-establishing AP2-O, a reduction of all but one gametocyte-related transcripts was observed. Surprisingly, reestablishment of AP2-O results also in the diversification of *var* transcripts, often silencing the previously active *var.* This was also paralleled by the loss of cytoadherence of infected red blood cells to specific receptors, since other PfEMP1s with different binding specificities became expressed. Our data point to the view that AP2-O has additional important regulatory roles in blood stage parasites and that the blockade of AP2-O may help in the elimination of specifically adherent parasite phenotypes. **Supported by:**FAPESP **Keywords:** Rif, var; antigenic variation; plasmodium falciparum

CO6 - **MOLECULAR GENETICS FOR CRYPTOSPORIDIUM**

STRIEPEN, B.*[1]
1.DEPARTMENT OF PATHOBIOLOGY, SCHOOL OF VETERINARY MEDICINE, UNIVERSITY OF PENNSYLVANIA, USA

The protozoan parasite Cryptosporidium is a leading cause of severe diarrhea in young children and an important contributor to early childhood mortality. Fully effective drugs and vaccines to treat or prevent cryptosporidiosis are lacking. A main roadblock for their development has the overall poor tractability of this parasite. We established a powerful molecular genetic model to overcome this hurdle. Using this system we can now engineer reporter parasites to derive robust measurements of infection in vitro and in vivo as well asparasite mutants to understand the mechanistic underpinnings of drug sensitivity and resistance in Cryptosporidium. Cryptosporidium is also a fascinating model to understand fundamental parasite biology. How does the parasite's complex sexual lifecycle unfold, and how do parasite, host and commensals interact to shape susceptibility, disease and protection? To address these issues we are pursuing different genetic strategies to identify key factors in the parasite and in the host. **Supported by: Keywords:** Cryptosporidium; genetics; apicomplexa

CO7 - **GENOME-WIDE STUDIES HELPING US TO UNDERSTAND DIFFERENTIATION AND INTRACELLULAR SURVIVAL OF TRYPANOSOMA CRUZI**

TEIXEIRA, S.M.R.*[1]
1.UFMG, MG, Brazil.

Trypanosoma cruzi, the causative agent of Chagas disease, is an intracellular protozoan pathogen that thrives within the cytoplasm of different types of mammalian cells such as macrophages, fibroblast, adipocytes, epithelial and muscle cells. To cope with its obligate intracellular lifestyle, T. cruzi had to develop molecular strategies to adapt to the host intracellular environment as well as to the mammalian bloodstream. As a parasite that is transmitted to its mammalian host by different species of insects belonging to the Triatominae family, T. cruzi also needs to adapt to the insect host environment. All of its adaptive strategies rely on complex regulatory mechanisms to modulate gene expression in each of its four main developmental forms, epimastigotes and metacyclic trypomastigotes (present in the insect vector), intracellular amastigote and bloodstream trypomastigotes (in the mammalian host). Being an early divergent eukaryote, T. cruzi has its genome organized in polycistronic transcription units, meaning that gene expression regulation occurs exclusively at the post-transcriptional level. RNA binding proteins (RBPs) are key post-transcriptional regulators that control processing, stability, translatability and localization of the mRNA population within the cell. Recent genome-wide expression studies have characterized the gene expression profiles throughout all the T. cruzi life cycle forms. These studies not only helped to unravel, at the molecular level, the most relevant processes involved in parasite interaction with its different hosts but also provided invaluable informations regarding the molecular mechanisms controlling gene expression as well as new targets for the development of more effective methods for disease control. We will discuss RNA-seq data that allowed us to identify molecular mechanisms involved with parasite differentiation, with its capacity to subvert host defense mechanisms and to manipulate host cell signaling pathways and the metabolic programming so that it can benefit of living in several distinct environments. We have also identified a regulatory RBP that control genes involved with parasite differentiation from mammalian to insect forms as well as an RBP that can suppress genes that are essential for parasite virulence.

Supported by:FAPEMIG - CNPq - INCTV
Keywords: Trypanosoma cruzi; gene expression ; rna-seq

CO8 - **DNDI - A NOVEL COLLABORATIVE R&D APPROACH TO DELIVER NEW TREATMENTS FOR NEGLECTED TROPICAL DISEASES**

KRATZ, J.*[1]
1.DNDI, RJ, Brazil.

The Drugs for Neglected Diseases initiative (DNDi) is a patients' needs driven, not for profit drug discovery and development organisation. Acting through a range of innovative and collaborative R&D models with partner organisations from around the globe, DNDi tackles the challenges of developing safe, effective and field adapted treatments for patients suffering from neglected diseases including sleeping sickness, leishmaniasis, Chagas disease, filarial infections, mycetoma, paediatric HIV infection, hepatitis C virus (HCV) infection, and is also developing the Global Antibiotic Research and Development Partnership (GARDP) to contribute to tackling issues of antimicrobial resistance.

Current drugs used for treating these diseases suffer from limitations in efficacy and safety (or high prices) and are not well adapted to the needs of patients. Therefore many patients are still in need of more effective, safer and convenient treatments. In recent years, new orally acting chemical entities have been designed and selected for development for treating visceral leishmaniasis, and perhaps also the cutaneous form of the disease. These new drug classes have been discovered using phenotypic drug discovery methods and offer great promise for developing new treatments, but their mechanisms of action are often not well understood. Efforts to de-convolute the mechanisms of action of these candidates and newer target based drug discovery approaches should open the door for discovery of further drug classes and candidate molecules.

This presentation will highlight the discovery of the NCEs now advancing in the clinic for leishmaniasis, and highlight the role of DNDi in boosting further R&D activities for neglected diseases in endemic areas. **Keywords:** Drug discovery; ntds; dndi

CO9 - **MEMBRANE PROTEINS INVOLVED IN IRON HOMEOSTASIS IN *LEISHMANIA***

LARANJEIRA-SILVA, M.F.*[1]
1.IB USP, SAO PAULO, SP, BRASIL.

Iron is essential for many cellular processes, but can generate highly toxic hydroxyl radicals in the presence of oxygen. Therefore, intracellular iron accumulation must be tightly regulated, by balancing uptake with storage or export. One mechanism used by a wide range of organisms to prevent iron toxicity is storage as a complex with ferritin. Other organisms manage iron accumulation by sequestering it in vacuoles. Iron acquisition in Leishmania is mediated by the coordinated action of two plasma membrane proteins, the ferric iron reductase LFR1 and the ferrous iron transporter LIT1. Leishmania can also acquire iron complexed to heme by LHR1. It was shown that the availability of iron regulates the expression of several genes in L. amazonensis and may trigger differentiation of the parasite to the intracellular amastigote stage. But besides all these studies, until recently it was still unclear how these parasites regulate their cytosolic iron concentration to prevent toxicity. Investigating this issue, we identified and characterized the Leishmania Iron Regulator 1 (LIR1), an iron responsive MFS-type plasma membrane protein with similarity to plant nodulin-like proteins. LIR1 localizes on the plasma membrane of L. amazonensis promastigotes and intracellular amastigotes. After heterologous expression in Arabidopsis thaliana, LIR1 decreases the iron content of leaves and worsens the chlorotic phenotype of plants lacking the iron importer IRT1. Consistent with a role in iron efflux, LIR1 deficiency does not affect iron uptake by L. amazonensis but significantly increases the amount of iron retained intracellularly in the parasites. Absence of LIR1 increases the sensitivity of Leishmania to iron toxicity and abolishes parasite infectivity. Given the essential role played by LIR1 in L. amazonensis iron homeostasis and virulence, and the lack of human orthologs, our findings suggest that LIR1 could be a promising target for the future development of therapeutic drugs. **Keywords:** Transporter; virulence; toxicity

CO10 - **SEARCHING NEW TARGETS FOR LEISHMANIASIS CHEMOTHERAPY BY FUNCTIONAL GENOMICS**

MURTA, S.M.F.*[1]
1.FIOCRUZ-MINAS, MG, Brazil.

Leishmaniasis represents a major public health problem worldwide. The high toxicity of drugs used in clinical treatment and the emergence of drug-resistant Leishmania parasites are challenges to successful treatment of disease. During some years our group has investigated the drug-resistance mechanisms in Leishmania spp. In a previous study, we selected in vitro lines of L. amazonensis, L. braziliensis, L. guyanensis and L. infantum resistant to potassium antimonyl tartrate (SbIII) (Liarte & Murta, 2010). Reduction in the intracellular levels of SbIII and lower expression of aquaglyceroporin 1, a membrane protein implicated in SbIII uptake, was observed in some of these SbIII-resistant Leishmania spp. lines (Moreira et al., 2013; Andrade et al., 2016).

Analysis of the proteome (Matrangolo et al., 2013), phosphoproteome (Moreira et al., 2015) and transcriptome (Andrade et al., in prep) showed that SbIII-resistance phenotype is complex, multifactorial, involves several pathways and differs among the species of Leishmania analyzed.

Functional analysis of enzymes involved in the antioxidant defense, trypanothione synthesis and other pathways showed that iron superoxide dismutase, tryparedoxin peroxidase, ascorbate peroxidase, pteridine reductase, nucleoside diphosphate kinase b, elongation factor 2, ornithine decarboxylase, gamma-glutamylcysteine synthetase and trypanothione synthetase are involved in the SbIII-resistance phenotype in Leishmania spp. (Andrade et al., 2014; Tessarollo et al., 2015; Moreira et al., 2015, 2016; Fonseca et al., 2017; Fonseca et al in prep). Compounds that inhibit some of these enzymes increased the antileishmanial effect of SbIII, being a valuable strategy for leishmaniasis chemotherapy (Moreira & Murta, 2016; Moreira et al., in prep).

Now we are using the Crispr/Cas9 genomic editing methodology to delete genes from important metabolic pathways in the search for new targets for chemotherapy of this important neglected disease.

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Keywords: Leishmania spp.; antimony-resistance; chemotherapy

CC1 - **MESSIEURS, C'EST LES MICROBES QUI AURONT LE DERNIER MOT." (GENTLEMEN, MICROBES WILL HAVE THE LAST WORD)**

VIEIRA, L.Q.*[1]
1.UFMG, MG, Brazil

What is the role of the host associated microbiota in response to infection with Leishmania? Our laboratory has, over the years, addressed this question. It had been established by MacDonald and Carter (1979) that germfree mice had a defective delayed type hypersensitivity. In the 90´s, it was made clear that a Th1-type response (which includes delayed type hypersensitivity) was necessary for the resistance to infection with Leishmania major in the murine model. Hence, we decided to investigate the course of infection with L. major in germ-free and conventional (microbiota-bearing) Swiss mice. Indeed, infection with L. major was more severe in germ-free mice, as measured by the size of infection (footpad) and number of parasites at the site of infection. However, when we measured the amount of inflammatory cytokines produced in draining lymph nodes and in the footpad

lesion, we found that IL-12, IFN-γ, and TNF were present in similar levels at all times of infection examined in both conventional and germ-free groups. Hence, there was not a Th1 bias conferred by the resident microbiota. There was, surprisingly, a higher amount of regulatory cytokines (IL-10 and TGF-β) at the site of infection in germ-free mice, even though lesions were larger. However, when we activated macrophages to kill parasites in vitro, we found that macrophages from conventional mice were more efficient in controlling parasites than macrophages from conventional mice. We then investigated the activation profiles of macrophages from conventional and germ-free mice. Conventional macrophages, when activated with IFN-γ and LPS (which would trigger killing of parasites) produced more IL-12 and TNF, and expressed higher message levels for NOS2, Socs1 and CXCL9 than macrophages from germ-free mice. In addition, conventional macrophages produced higher levels of reactive oxygen species and NO. On the other hand, when activated with IL-4 or PGE2 and LPS, macrophages from germ-free mice expressed higher levels of wound-healing and regulatory markers. Hence, it seems that the microbiota instructs macrophages to respond to inflammatory stimuli more readily and intensively. Accordingly, when the microbiota was decreased drastically by antibiotic treatment, antibiotic-treated conventional macrophages behaved similarly to germ-free macrophages. In vivo, we could not find differences in the numbers of T cells in lesions, but the number of CD11b+ cells was higher in lesions from germ-free mice at the later time points of infection. Of these CD11b+ cells, monocytes, macrophages and neutrophils and a yet undetermined population were more infected with L. major in germ-free lesions. Macrophages isolated from lesions from conventional mice expressed more NOS2, in accordance with our in vitro data. Finally, subtraction of the microbiota by antibiotic treatment also changed the course of infection in conventional mice towards the outcome in germ-free mice. In conclusion, the microbiota seems to train the innate immune system towards an inflammatory phenotype. **Supported by:**CAPES, CNPq, FAPEMIG and NIH **Keywords:** Germ-free; macrophages; leishmania