Samuel Pessoa Conference

SPC001 - BINDING TO THE EXTRACELLULAR MATRIX INDUCES BIOCHEMICAL CHANGES IN *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES

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The infective trypomastigote forms of *Trypanosoma cruzi* employ a repertoire of molecules that bind to different host receptors. Our group described earlier in trypomastigotes an 85 kDa molecular mass surface glycoproteins (named Tc-85) that is involved in host cell infection, since a monoclonal antibody raised against Tc-85 inhibited *in vitro* infection by 50-70%. Later on, Tc-85 has been classified as a member of group II of the gp85/trans-sialidase family. It has also been found that Tc85 binds to laminin in a carbohydrate independent way, with the binding site located at the amino-terminal domain of the molecule. In addition, our group has also shown that Tc-85 recombinant proteins are multiadhesive since they are able to bind to prokineticin receptors, as well as to laminin, fibronectin and cytokeratins (Khusal K, et al . Parasitol Res.114, 155, 2014; Mattos EC et al. Subcell Biochem 74, 151, 2014). These results contribute to the general assumption that the parasite population possesses an extensive repertoire of molecules involved in the infection of almost all host cells and that these molecules, at least in part, are multiadhesive proteins. Additionally, it was described for the first time that laminin, a component of the extracellular matrix (ECM), is important to the mammalian infection (rev. Mattos EC et al. Subcell Biochem 74, 151, 2014).

ECM is a very dynamic structure, composed of hundreds of molecules, which in association with extracellular regulatory molecules, can be defined as the extracellular environment. It is now well established that trypomastigotes interact with the three major structural components of ECM: proteoglycans, collagen fibers and multiadhesive proteins (such as laminin and fibronectin) (rev. Mattos EC et al. Subcell Biochem 74, 151, 2014). What would be, then, the possible cellular responses of trypomastigotes when interacting with ECM, an obligatory step in infection?

Since protein post-transcriptional modifications are common responses to different stimuli, our studies have been focused on phosphorylation, nitration and S-nitrosylation of trypomastigote proteins upon treatment with ECM. As a general result, a down regulation of these three modifications was observed in ECM-treated trypomastigotes, as detected by proteomic and immunochemical analysis.

From the 303 phosphopeptides identified in phosphoproteomic studies, approximately 23% were phosphorylated, while 77% were dephosphorylated in ECM-treated parasites as compared to untreated parasites. Of these, 67% correspond to hypothetical proteins, 11% to phosphopeptides from cytoskeletal proteins, and ca. 5% to kinases and phosphatases. Also, heat shock proteins and proteins from the intermediate metabolism have been identified (Eliciane Mattos et al, unpublished; Mattos EC et alPlos One 7, e46767, 2012).In addition to the general down regulation of the S-nitrosylation and nitration modifications in proteins of ECM-treated parasites, a decrease in both NOS activity and cGMP concentration have also been detected. However, some specific proteins, such as enolase and mucin TcMUCII had, respectively, their nitration and S-nitrosylation levels increased, suggesting that both modifications do not follow the decrease in NOS activity, thus possibly reflecting the action of other signaling pathways in *T.cruzi* (Pereira MA et al, Plos Negl. Trop. Dis. 9, e0003683, 2015).Overall, evidence presented here points to the direction of a massive down regulation of protein phosphorylation, nitration and S-nitrosylation when trypomastigotes interact with ECM. This is probably connected with changes in metabolism, in preparation for the next intracellular environment that the parasite will have to face.

Supported by:FAPESP, CNPq, CAPES **Keywords:**Trypanosoma cruzi ; trypomastigotes; ecm

CO.01 - ADIPOSE TISSUE AND PATHOGENESIS OF CHAGAS DISEASE

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Adipose tissue is no longer regarded as an inert organ whose only purpose is triglyceride storage. In fact, it is the largest endocrine organ in the body and is important in a variety of physiological functions. Adipose tissue and the adipocyte are early targets of *Trypanosoma cruzi* infection and likely enter the cells via the Low Density Lipoprotein Receptor. Adipose tissue is likely to be the major reservoir for the parasite in chronic Chagas disease, as we found it to be persistent in adipocytes in both chronically T. cruzi-infected mice and in patients. Long-term infection of adipose tissue markedly alters its function, and all the pathological changes observed during the chronic stage of the infection are reminiscent of morbid obesity. Numerous studies have examined the changes in adipose tissue initiated by *T. cruzi* infection. Within 10 to 15 days post *T. cruzi* (Brazil strain) infection, there is a significant increase in proinflammatory mediators and a migration of F4/80-positive macrophages into the inflamed adipose tissue. This is accompanied by a reduction in the levels of the anti-inflammatory adipokine adiponectin, both in adipose tissue and blood. *T. cruzi* Infection of cultured adipocytes also results in increased expression of proinflammatory mediators. As the infection becomes more chronic, there is a persistent continued increase of proinflammatory mediators in adipose tissue. Oxidative stress is a marked consequence of *T. cruzi* infection of both brown and white adipose tissues. When lipid peroxidation and carbonylation were examined in CD-1 mice at 15, 30, and 130 days post infection, markers of oxidative stress were upregulated in both tissues at all time points. Determinants of anti-oxidative stress were downregulated in parallel. This increase in oxidative stress during *T. cruzi* infection most likely has a deleterious effect on host metabolism and on the heart. We have also examined the gap junction protein connexin 43 (Cx43) expression in infected adipose tissues. Although gap junctions are a prominent feature of brown adipocytes, they have not been explored extensively in white adipocytes, especially in the setting of infection. We examined the functional coupling in both white and brown adipocytes in mice. Injection of the dye Lucifer Yellow into adipocytes within fat tissue spread to adjacent cells and was reduced by treatment with agents known to block gap junctions. Cx43 was detected in both brown and white fat tissue. At thirty and ninety days post-infection, Cx43 was downregulated in brown adipocytes, however, dramatically upregulated in white adipocytes. Therefore, we conclude that gap junction-mediated intercellular communication contributes to the cellular response to infection in the adipocyte. We also found that Cx43 upregulation in particular in the inguinal fat pad may drive “beige” adipogenesis involved in heat production. Thus, adipose tissue and the adipocyte specifically are significant players in the pathogenesis of Chagas disease, both as an important target cell during the initial infectious cycle as well as a reservoir for parasites in the chronic stage. Adipocytes may, therefore, be an important source of re-emerging parasites during Chagas disease reactivation.

Keywords: Adipocytes; trypanosoma cruzi; gap junction

CO.02 - GENETIC ANALYSIS OF VIRULENCE IN TOXOPLASMA GONDII

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Toxoplasma gondii is a widespread parasite of animals that also causes opportunistic infections in humans. The population structure of *T. gondii* is highly unusual with three clonal lineages that predominate in North American and Europe. In contrast, strains in South America and much more diverse and undergo frequent genetic exchange. Despite these differences in population structure, previous genetic mapping studies have demonstrated that same parasite factors control the virulence of *T. gondii* strains in the mouse model. Acute virulence is largely mediated by a family of polymorphic kinases that are secreted into the host cell at the time of invasion. The rhoptry kinases ROP18 and ROP17, along with the pseudokinase ROP5, control acute virulence by targeting and disabling immunity related GTPases that are upregulated by interferon gamma. As well, *T. gondii* secretes a soluble mediator that binds to STAT1 and blocks its transcriptional activity by recruiting a repressive chromatin-modifying complex. Finally, a host non-canonical autophagy pathway contributes to innate control of infection, yet virulence strains have adaptations that allow them to avoid this restrictive pathway. Collectively, these studies reveal a diversity of parasite adaptations that block innate immunity and therefore contribute to pathogenesis in rodents and in humans.

Keywords: Rhoptry kinase; irgs; non-canonical autophagy

CO3 - ENDOGENOUS RNA VIRUSES AS VIRULENCE FACTORS IN PARASITIC PROTOZOA

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Virus-like elements occur in many parasitic protozoans, but previously were seen largely as molecular curiosities of uncertain importance. *Leishmania* in South America often bear the dsRNA *Leishmaniavirus* (LRV1). Like most Totiviruses, LRV1 is neither shed nor infectious, and thus may be viewed as a persistent endobiont. Perspectives on the importance of these elements changed upon discovery that *L. guyanensis* LRV1 is associated with hypervirulence and increased metastasis, the latter being a hallmark of the more severe forms of leishmaniasis (Ives *et al. Science* 2011). We have been pursuing this observation intensively as a new paradigm of protozoal virulence. For *Leishmania* we developed tools for reproducibly generating isogenic lines lacking LRV1s. This has allowed extension of findings with *L.guyanensis* to *L. braziliensis*, the predominant agent of mucocutaneous leishmaniasis (MCL). Transcriptomic analysis of infected macrophages shows an elevated 'hyperinflammatory' response including stimulation of many Type I interferon-inducible genes. Another question is the contribution of LRV1 with *Leishmania* pathogenicity in human infections, where disease manifestations differ greatly from those seen in murine models, which is complicated by several factors. Recently we showed that the presence of LRV1 was associated with increased relapse and/or treatment failures in human *L.braziliensis*-infected patients treated with pentavalent antimonials in Peru and Bolivia, as well as in *L.guyanensis* infections treated with pentamidine (Adauai *et al* & Bourreau *et al. J. Inf. Dis* 2016). The association of LRV1 with clinical drug treatment failure could serve to guide more effective treatment of tegumentary disease caused by *Leishmania* sp.

I thank my many collaborators and colleagues in the references cited above, especially the members of my laboratory and Nicolas Fasel and colleagues at the University of Lausanne, Switzerland. Supported by US NIH grants.
Keywords: Leishmania; rna viruses; virulence

CO4 - **COMPLEX PARASITE PROBLEMS OR SIMPLE MINDS?**

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The last 35 years in parasitology are marked, among other aspects, by an intense exploration of the host immune response against parasites. Since those early days, such an endeavor was based on the assumption that understanding host immune response would lead us to develop diagnostic tests and anti parasitic vaccines. Although we have developed considerably better diagnostic tests in this period, the development of a vaccine against any parasite is far from satisfactory. Even conceding that 35 years is a minuscule time lapse compared to the long journey that parasites and mankind have shared, reviewing the attempts for developing a comprehensive view of the human immune system may help in preparing for the enduring challenge ahead of us.

The presentation will review the early steps of groping in the dark represented by lymphocyte proliferation tests performed in the 70's and 80's of the 20th century to the modern magical present days of RNA -seq, all based on the personal experience of the author.

Keywords: Parasites; immune response; complexity