Sunday, 01 May 2011

THE GENUS *PARACOCCIDIOIDES*: FROM VISUAL OBSERVATIONS TO GENOMICS IN ONE HUNDRED YEARS

Author: GIOCONDA SAN-BLAS [1]
Institution: 1. IVIC; Instituto Venezolano de Investigaciones Científicas

Abstract:
Paracoccidioides brasiliensis, the causative agent of paracoccidioidomycosis (PCM) was first reported by Adolpho Lutz in São Paulo, 1908. PCM is one of the most frequent systemic mycoses reported in South America, particularly Brazil, Colombia and Venezuela. Until the beginning of the 6th decade of the past century, research on PCM was mainly restricted to clinical aspects of the disease, although morphological observations started from the very beginning, Lutz himself describing the dimorphic character of the fungus. From the late 1960s onwards, early microscopic research on the biology of *P. brasiliensis* scaled up to electron microscopic observations on its ultrastructure, followed by chemical studies on cell wall composition, which provided initial evidence of chemical changes involved in both the dimorphic process and the pathogenicity of *Paracoccidioides*. Immunological aspects of the disease have also been studied at large, providing a wealth of information that has been instrumental in clinics. These studies, however, will not be covered in this conference. The development of molecular tools for the study of biological systems resulted in the incorporation of them to further studies on *P. brasiliensis*, from the 1970s onwards. With them, phenomena such as dimorphism, virulence factors, immunology of the disease, development of potential vaccines, and many others have reached a stage of sophistication hardly foreseen by early researchers in the field. Additionally, the discovery of molecular species within the genus *Paracoccidioides* (the cryptic species S1, PS2 and PS3, and *Paracoccidioides lutzii*) may help in the understanding of epidemiological, immunological, and clinical features that have remained obscure to this day. Furthermore, a continental effort carried out by the Broad Institute (MIT, Boston, Mass.) together with all main Latin American laboratories involved in molecular aspects of the genus, led to the decoding of three *Paracoccidioides* genomes, two of them (Pb03 and Pb18) *P. brasiliensis* and one (Pb01) *P. lutzii*. In this conference, we intend to discuss some of the most relevant aspects of recent biological research on the genus *Paracoccidioides*, and provide ideas for future lines of investigation.

Keywords: *Paracoccidioides*, biology, molecular, speciation, biochemistry

Monday, 02 May 2011

THE ROLE OF IL-18 IN *PARACOCCIDIOIDES BRASILIENSIS* ANTIFUNGAL ACTIVITIES OF HUMAN MONOCYTES

Author: DIAS-MELICIO, L.A. [1], SOARES, A.M.V.C. [1]
Institution: 1. UNESP- IB; Universidade Estadual Paulista

Abstract:
In recent years, our group has evaluated the mechanisms involved in the interaction of *P. brasiliensis* with human phagocytic cells that results in fungus killing. Interleukin -18 (IL-18) is a cytokine that contributes to host defense infection by increasing antimicrobial function of phagocytes and initiating TH1 and TH17 adaptative immune responses. The role of this cytokine in paracoccidioidomycosis is not fully understood, and to date there are no studies on its role in antifungal activities of phagocytic cells. In this context, we assessed the action of IL-18 on human monocytes activities against *P. brasiliensis* by evaluating fungicidal activity and H2O2 release. In addition, we studied IL-18 capacity to modulate TLR2, TLR4 and mannose receptor (MR) expression, as well as IL-18, IL-10, TNF-alfa, IL-12 and IL-15 production by these cells. Unexpectedly, the results showed that IL-18 increased fungal recovery from infected monocytes. Accordingly, H2O2, a metabolite involved in P.brasiliensis killing, was not affected. In addition, IL-18 increases MR and TLR4 expression and TNF-alfa and IL-10 production by monocytes. Blocking assays revealed that IL-18 role in *P. brasiliensis* growth in human monocytes is mediated by MR, while increase in TNF-alfa and IL-10 levels was associated with TLR4 and TLR2 respectively. Our data strongly suggest that, although IL-18 was able to induce high levels of pro and anti-inflammatory cytokines, which potentially could act as modulators of phagocytic cells activity, the effect of IL-18 on the increase of fungal recovery on monocytes, would be due to capacity of this cytokine to modulate MR expression. We are hypothesizing that increase on MR expression in monocytes, by IL-18, will both, facilitate the entry of the fungus in the cell, and activate a new molecular pathway involving peroxisome proliferator-activated receptor (PPARγ)activation, which regulates the macrophage inflammatory response, thereby playing a role in paracoccidioidomycosis pathogenesis. Finnancial support: FAPESP and CNPq

Keywords: antifungal activity , IL-18 , mannose receptor , monocytes, *Paracoccidioides brasiliensis*

***PARACOCCIDIOIDES BRASILIENSIS*-INDUCED MIGRATION OF RESPIRATORY DENDRITIC CELLS AND SUBSEQUENT ANTIGEN PRESENTATION IN THE LUNG DRAINING LYMPH NODE**

Author: ALMEIDA SR [1]
Institution: 1. USP; Universidade de Sao Paulo

Abstract:
Paracoccidioidomycosis is a mycotic disease caused by a terminally dimorphic fungus, *Paracoccidioides brasiliensis* (Pb), that starts with inhalation of the fungus; thus, lung cells such as DC are part of the first line of defense against this microorganism. Dendritic cells (DC) are antigen-presenting cells that act as sentinels in peripheral tissues, constantly sampling the antigens in their environment. Lung DC play a pivotal role in infections caused by airborne pathogens such as Mycobacterium tuberculosis, Aspergillus fumigatus and Cryptococcus neoformans. The DC population lining the lungs is key to the initiation of T cell responses after pulmonary challenge. DC remain quiescent until activated, at which point they migrate to the draining lymph nodes (DLN), present antigen, and initiate T cell activation. Because DC are the most effective antigen presenting cells (APC) for inducing cell-mediated immune responses, it is important to investigate lung DC and their potential for lymph node migration and immune response initiation. The mechanisms involved in resistance to Pb infection are poorly understood, but it is likely that DC play a pivotal role in the induction of effector T cells that control Pb infection. In this study, we analyzed the role that DC played in modulating Pb infection. We observed an increased expression of CCR7 and CD103 on lung DC after infection, as well as MHC-II. After Pb infection, bone marrow-derived DC as well lung DC, migrate to lymph nodes. Migration of lung DC could represent an important mechanism of pathogenesis during PCM infection. In resume our data showing that Pb induces migration of DC. Furthermore, we demonstrated that bone marrow-derived DC stimulated by Pb migrate to the lymph nodes and activate a T helper (Th) response. Elucidating the mechanisms of regulation and coordination of DC migration by pathogen-derived signals requires more study. An understanding of the signals that regulate DC migration could allow for the development of methods to induce efficient T-cell activation that would aid in the control of PCM infection.

Keywords: Dendritic cell, lung, T cell

THE ROLE OF NK CELLS IN HUMAN PARACOCCIDIOIDOMYCOSIS

Author: RONEI LUCIANO MAMONI [1]
Institution: 1. UNICAMP; Universidade Estadual de Campinas

Abstract:
Besides their role in viral infection and tumor resistance, recent studies have showed that NK cells also participate in the immune response against other infectious diseases. The aim of this study was to evaluate the possible role for NK cells (CD56+ cells) in the immune response against the dimorphic fungus *Paracoccidioides brasiliensis*. It was found that NK cells from patients present a lower citotoxic response when compared to healthy individuals. The analyses of CD56+ cells from controls showed an elevated expression of CD25 and CD69 after the stimulus with yeast cells, while cells from patients are activated only in the presence of IL-15. Furthermore, our results showed that CD56+ cells are able to directly recognize and kill *P. brasiliensis* yeast cells, and that this activity seems to be granule-dependent but perforin-independent, while the citotoxicity against monocytes infected with *P. brasiliensis* showed to be perforin-dependent. Infected monocytes augmented their expression for IL-15 mRNA, while exhibit a diminished number of MHC class I molecules, associated with an increased expression of MICA/B molecules in their surface. It was also observed an augmented expression of granulysin in NK cells from healthy individuals, and that CD56+ cells are able to produce and release granulysin after stimulation with *P.brasiliensis*. One possible mechanism for the recognition of *P. brasiliensis* by NK cells is through the TLR2, TLR4 and CR3. We observed that NK cells express TLR2 and CR3 on their surface, and that IL-15 increases the expression of TLR2. These results demonstrated that CD56+ cells can actively participate in the immune response against the *P. brasiliensis* infection either by directly destroying yeast cells or by the recognition and killing of infected cells. Granulysin is the possible mediator of the cytotoxic effect observed in this study, once this protein is produced and released by CD56+ cells. Furthermore, our data showed that NK cells express TLR2, TLR4 and CR3, which may be responsible for the recognition of yeast cells through the beta-glucan present in the yeasts cell-walls. Another important data is the finding that CD56+ cells are able to produce IFN-gamma and TNF-alpha, which could influence the subsequent acquired immunological response by stimulating other cells as dendritic cells, macrophages and lymphocytes.

Keywords: Granulysin, NK cells, TLR2, Paracoccidioidomycosis

IMMUNE RESPONSE IN HUMAN PARACOCCIDIOIDOMYCOSIS FROM INFECTION TO "CURE"

Author: SATO, P.K. [1], FURUCHO, C.R. [1], SADAHIRO, A. [2], DIOGO, C, L. [1], SHIKANAI-YASUDA, M.A. [1]
Institution: 1. Fac. Medicina USP; Laboratorio de Imunologia, LIM 48, Hospital das ClInicas
2. UFAM; Universidade Federal do Amazonas

Abstract:
Cellular immune response represents a major expression of defence in human paracoccidioidomycosis (PCM). The Th1-type of immune response plays an important protective role in the resistance to *P. brasiliensis* (Pb) and its cytokine profile has been associated to PCM infection with no clinical symptoms. Comparative studies in infected and non infected relatives (RE) of patients with PCM in our laboratory suggest that some infected individuals of RE group produced high levels of IFN-γ in contact to Pb antigens, suggesting that they are able to control the fungus replication after the exposition sensitization in comparison with treated chronic cases. The Th2 cytokine profile has been related to the acute PCM and patients with disseminated disease presented imbalance in cytokines profile and transitory immunodepression in response to Pb immunodominant component, the 43kDa glycoprotein (gp43), recovering after clinical cure. Concerning genetic factors, we showed that DRB1*11 is more frequent in patients with the unifocal chronic form of the disease, a mild clinical presentation in which lesions are restricted or localized. In human PCM, we investigated the expression of surface molecules and the production of cytokines by monocyte derived DCs from patients with active or cured PCM. DCs from the treated group stimulated with either 43kDa glycoprotein (gp43) or cell-free antigen(CFA) from Pb showed higher expression of HLA-DR and CD86 and higher levels of IL-12p40 compared to healthy controls, whereas active disease group showed similar expression to the control group. Production of IL-10 was up-regulated by gp43 and TNF-α only on the treated group when compared to DCs treated with TNF-α alone.CFA induced a stronger autologous lymphoproliferation and higher levels of IFN-γ and TNF-α on treated and active disease groups compared to control group. CFA may be a suitable antigen to induce cellular response through DCs antigen presentation even on active PCM patients. Higher expression of surface molecules with increased IL-12p40 may indicate a better activation of DCs after treatment of PCM, In fact, DCs may be crucial in the protective response to Pb. Adittionally, in vitro-generated DCs might be useful in enhancing antifungal immunity, especially during active PCM. Financial support: FAPESP 2004/14955-3,F.Medicina Foundation, Hosp. Clinicas Faculdade Medicina - USP.

Keywords: HLA DR/DQ, paracoccidioidomycosis, infection and , dendritic cells, cytokines

CRYPTIC SEX IN THE FUNGAL PATHOGEN CANDIDA ALBICANS

Author: PROF. BENNETT, R.J[1]
Institution: 1. Brown; Brown University

Abstract:
Our laboratory studies the opportunistic pathogen *Candida albicans*, as well as the closely related species *C. tropicalis* and *C. parapsilosis*. These fungi are hemiascomycete yeast that are usually harmless commensals in the human body, but are also capable of causing life-threatening bloodstream infections. The focus of our research is the mechanism of sexual reproduction in *Candida* species, and the potential roles of these pathways in promoting infection and pathogenesis. One striking feature of mating in *C. albicans* is that it is regulated by the white-opaque phenotypic switch. *C. albicans* **a** and alpha cells can undergo reversible and heritable switching between white and opaque states. Cells in the opaque state are competent for mating, whereas cells in the white state are more virulent in bloodstream infections but are incapable of mating. Pheromone signaling between opaque **a** and opaque alpha cells coordinates the mating process, resulting in tetraploid mating products. We recently demonstrated a novel mode of self-fertilization in *C. albicans* whereby opaque **a** cells could self-mate due to autocrine pheromone signaling. Furthermore, we show that pheromones from one *Candida* species are active in inducing same-sex mating of a different *Candida* species. Inter-species signaling could therefore be important in driving sexual mating events in nature. Finally, we are interested in how mating genes can regulate processes important for colonization and infection. One striking example of this is that cell surface factors induced during the mating process can promote adhesion and biofilm formation, a critical step in the development of device-associated infections. We therefore discuss the broader roles of pheromone-signaling, phenotypic switching, and sexual reproduction in the context of fungal pathogenesis and disease.

Keywords: Mating, Phenotypic Switch, Sexual Reproduction

MOLECULAR AND MORPHOLOGICAL ASPECTS OF MATING IN PARACOCCIDIOIDES: COMPARATIVE STUDIES WITH OTHER DIMORPHIC FUNGAL PATHOGENS

Author: MARCUS TEIXEIRA [1]
Institution: 1. UnB; Universidade de Brasilia

Abstract:
The genus Paracoccidioides includes the thermodimorphic species *P. brasiliensis* and P. lutzii, both of which are the etiological agents of paracoccidioidomycosis, a systemic mycosis that affects humans in Latin America. Phylogenetically, Paracoccidioides may be located in the family Ajellomycetaceae, together with other fungal pathogens. Despite the common occurrence of a sexual stage among the members of this family, this has not been observed with Paracoccidioides species, which have thus been hitherto considered asexual. Molecular evolutionary studies revealed recombination events within isolated populations in the genus Paracoccidioides, suggesting the possible existence of a sexual cycle. Comparative genomic analysis of dimorphic fungi and Saccharomyces cerevisiae demonstrated the presence of conserved genes involved in sexual reproduction, including mating regulators such as the MAT locus, α pheromone precursor gene, pheromone receptors, pheromone processing enzymes and mating signaling regulators. The expression of sex-related genes in the yeast and mycelial phases of both Paracoccidioides species was also detected by real-time polymerase chain reaction, with nearly all of these genes being preferentially expressed in the filamentous form of the pathogens. In addition, the expression of sex-related genes was responsive to the putative presence of pheromone in the supernatants obtained in previous co-cultures of two different mating-type strains. In vitro crossing of different mating-type isolates, discriminated by phylogenetic analysis of α-box (MAT1-1) and HMG (MAT1-2), led to the identification of the formation of young ascocarps with constricted coiled hypha related to the initial stage process of mating. These genomic and morphological analyses strongly support the existence of a sexual cycle in species of the genus Paracoccidioides. Funding: CNPq/FAP-DF

Keywords: speciation, mating, *Paracoccidioides brasiliensis*, Paracoccidioides lutzii

PRESENCE AND EXPRESSION OF THE MATING TYPE LOCUS IN *PARACOCCIDIOIDES BRASILIENSIS* ISOLATES.

Author: TORRES I [1,3], MCEWEN JG [1,2], RESTREPO A [1], ARANGO M [1,2]
Institution: 1. BCM, CIB; Cellular and Molecular Biology Unit, CIB, Medellin, Colombia
2. U de A, Medellin, Co; Facultad de Medicina, U de Antioquia, Medellin, Colombia
3. U de A, Medellin, Co; Biology Institute

Abstract:
In fungi, sexual reproduction is regulated by a group of genes known as the Mating Type locus (MAT 1). In *Paracoccidioides brasiliensis*, an important human pathogen, sexual reproduction has not been demonstrated and consequently, its implications in fungal taxonomy and virulence remain undefined. In this work, the presence of both MAT 1 genes (MAT1-1 and MAT1-2) in a group of 71 *P. brasiliensis* isolates from diverse sources was ascertained: in some, the MAT 1 gene activity was determined by measuring the corresponding expression by qPCR. Additionally, a number of outcrosses among compatible isolates were explored. We found two heterothallic groups, one carrying a MAT1-1 gen and another carrying a MAT1-2 gene. These populations were in a 1:1 ratio with basal gene expression being detected for certain of these genes, indicating that the mating process could be feasible. Some of the crosses performed on special culture media displayed a number of structures resembling fruiting bodies, although they did not contain the expected reproductive structures (asci and ascospores) corresponding to the Ascomycota phylum. Demonstration of the presence of the MAT1-1 and MAT1-2 genes in *P. brasiliensis* has provided new tools for the study of sexual reproduction in this fungus, leading to a better understanding of its biology both as a saprobe and as a pathogen, as well as to more precise taxonomic classification. Further studies should be conducted to confirm the sexual capacity of this fungus and its implications among phylogenetic species and geographical distribution. This work was supported by a grant from Banco de la República de Colombia, proyect No. 2.051 and counted with with the collaboration of Drs. Joseph Heitman and Wenjun Li from the Center for Microbial Pathogenesis, Department of Molecular Genetics and Microbiology (MGM), Duke University Medical Center, Durham, NC.

Keywords: *Paracoccidioides brasiliensis*, Mating type, MAT 1-1, MAT1-2, Real time PCR

PRP8 INTEIN: A PARASITIC GENETIC ELEMENT IN A HOUSE KEEPING GENE OF *PARACOCCIDIOIDES BRASILIENSIS*- MEANING AND APPLICATIONS

Author: THEODORO, R.C. [1], BAGAGLI, E. [1]
Institution: 1. UNESP; Universidade Estadual Paulista Júlio de Mesquita Filho

Abstract:
Inteins are intervening sequences translated in frame with the host protein coding gene. They are self-excised through protein splicing, joining the N and C terminals of the host protein by a peptide bond, maintaining its normal function. Besides their splicing domain, inteins might also contain a homing endonuclease (HE) domain, making them mobile elements. Inteins are also considered promising therapeutic drug target, since once the autocatalytic splicing is inhibited, the host protein, which is typically vital, will not be able to perform its normal function and the fungal cell will not survive or reproduce. The PRP8 intein occurs in the Prp8 protein, a component of U5 snRNPs implicated in the editing of pre-messenger RNAs. This intein has been found in important human pathogens from *Cryptococcus* and *Aspergillus* genera and also from Ajellomycetaceae family, such as *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Emmonsia parva* and Paracoccidioides species. The phylogenetic potential of this region was studied in order to distinguish the four cryptic species of *Paracoccidioides* genus (S1, PS2 and PS3, from the *P. brasiliensis* complex, and *P. lutzii*). Maximum-Parsimony, Maximum Likelihood, and Bayesian analysis clearly separated the isolates from the three species and revealed a significant divergence between the Pb01 isolate, from *P. lutzii* species, and the remaining ones. The splicing function of the PPR8 inteins from B. dermatitidis, E. parva and four cryptic species of *P. brasiliensis* was evaluated in a non-native recombinant protein context by inserting the intein between a Maltose Binding Protein and a Thioreodoxin, in *Escherichia coli* cells. All PRP8 inteins proved to be active in this extein model, suggesting their usefulness for screening drugs that inhibit the intein excision in pathogens from the Ajellomycetaceae family. In silico and in vitro experimental analyses of HE domain provide evolutionary evidence that the *P. brasiliensis* PRP8 intein lost its endonuclease activity, revealing that this domain was active until recently but it is now in a degenerative process. Financial Support: Fapesp 2007/01306-5 and 2010/08829-6.

Keywords: Ajellomycetaceae, cryptic species, endonuclease domain, splicing domain, therapeutic target

IDENTIFICATION OF TRANSPOSABLE ELEMENTS IN GENOMES OF *PARACOCCIDIOIDES* SPECIES COMPLEX

Author: CISALPINO, PS [1]
Institution: 1. UFMG; Universiade Federal de Minas Gerais

Abstract:
The genetic polymorphism of Paracoccidioides has been widely demonstrated by different authors using several molecular techniques. The effects of transposable elements (TEs) may be responsible for the genetic polymorphism observed in fungal genomes. Studies based on gene genealogies suggested that the genus consists of at least four phylogenetic species, S1 (*P. brasiliensis*), PS2, PS3, and a quite distinct lineage referred to as Pb01-like, now proposed to be a new species, P. lutzii. Recently, three isolates corresponding to distinct Paracoccidioides phylogenetic lineages had their genomes sequenced. Our group carried out a genomic survey for the identification and characterization of class II transposable elements (DNA transposons) in their genomic sequence assemblies. Eight new Tc1/mariner families, referred to as Trem (Transposable element mariner), labeled A - H were identified. Elements from each family have 65-80% sequence similarity with other Tc1/mariner elements. They are flanked by 2-bp TA target site duplications and different termini. Encoded DDD-transposases, some of which have complete ORFs, indicated that they could be functionally active. The distribution of Trem elements varied between the genomic sequences characterized as belonging to *P. brasiliensis* (S1 and PS2) and P. lutzii. TremC and H elements would have been present in a hypothetical ancestor common to *P. brasiliensis* and P. lutzii, while TremA, B and F elements were either acquired by *P. brasiliensis* or lost by P. lutzii after speciation. Although TremD and TremE share about 70% similarity, they are specific to *P. brasiliensis* and P. lutzii, respectively. This suggests that these elements could either have been present in a hypothetical common ancestor and have evolved divergently after the split between *P. brasiliensis* and P. lutzii, or have been independently acquired by horizontal transfer. Families were distinguished based on significant BLAST identities between transposases and/or TIRs. The expansion of Trem in a putative ancestor common to the species *P. brasiliensis* and P. lutzii would have given origin to TremC and TremH, while other elements could have been acquired or lost after speciation had occurred. The results may contribute to our understanding of the organization and architecture of genomes in the genus Paracoccidioides.

Keywords: DNA tranposons, Paracoccidioides, class II transposons

COMPARATIVE GENOMICS OF PARACOCCIDIOIDES AND GENE FAMILY EVOLUTION IN THE DIMORPHIC FUNGI

Author: DESJARDINS, C. A. [1]
Institution: 1. Broad Institute; Broad Institute

Abstract:
Paracoccidioides is a fungal pathogen and the cause of paracoccidioidomycosis, a health-threatening human systemic mycosis endemic to Latin America. Infection by *Paracoccidioides*, a dimorphic fungus in the order Onygenales, is coupled with a thermally regulated transition from a soil-dwelling filamentous form to a yeast-like pathogenic form. To better understand the genetic basis of growth and pathogenicity in *Paracoccidioides*, we sequenced the genomes of two strains of *P. brasiliensis* (Pb03 and Pb18) and one strain of *P. lutzii*. These genomes range in size from 28.8 to 32.9 Mb and encode 7,610 to 8,130 genes. To enable genetic studies, we mapped 94% of the *P. brasiliensis* Pb18 assembly onto five chromosomes. We characterized gene family content across Onygenales and related fungi, and within *Paracoccidioides* we found expansions of the fungal-specific kinase family FunK1. Additionally, the Onygenales have lost many genes involved in carbohydrate metabolism and fewer genes involved in protein metabolism, resulting in a higher ratio of proteases to carbohydrate active enzymes in the Onygenales than their relatives. To determine if gene content correlated with growth on different substrates, we screened the non-pathogenic onygenale *Uncinocarpus reesii*, which has orthologs for 91% of *Paracoccidioides* metabolic genes, for growth on 190 carbon sources. U. reesii showed growth on a limited range of carbohydrates, primarily basic plant sugars and cell wall components, suggesting that Onygenales, including dimorphic fungi, can decay simple plant biomass in the soil. By contrast, *U. reesii* showed extensive growth on a wide range of dipeptides and amino acids, indicating a ability to utilize proteinaceous growth substrates, and suggesting that these fungi can also degrade animal biomass. The ability to utilize a wide range of proteins coupled with the evolutionary conservation of protease diversity may have predisposed the dimorphic fungi, including *Paracoccidioides*, to a pathogenic lifestyle on a live animal host.

Keywords: genomics, metabolism, kinases

LESSONS FROM DISTANT RELATIVES: SYSTEMS BIOLOGY OF KINETOPLASTIDS AS A MODEL FOR DISCOVERY OF THERAPEUTIC TARGETS IN PATHOGENIC FUNGI

Author: IGOR C. ALMEIDA [1]
Institution: 1. UTEP; University of Texas at El Paso

Abstract:
Pathogenic kinetoplastids, such as the protozoan parasites *Trypanosoma cruzi* and *Trypanosoma brucei*, are responsible for major infectious diseases, namely Chagas disease (CD) and human African trypanosomiasis (HAT), which affect millions of people worldwide and cause thousands of deaths and significant economic losses in developing countries. Current drugs for treating these diseases are partially effective and highly toxic, and vaccines are not available. Thus, there is an urgent need for the discovery of molecular targets for the development of new chemo- and immunotherapeutic interventions. In this regard, our group and several collaborators have been developing novel systems biology (omics) approaches and adapting established ones (e.g., proteomics, lipidomics, glycomics) for the global analysis of proteins, lipids, glycolipids, and co-/post-translational modifications (CTMs/PTMs) (e.g., glycosylation, GPI-anchoring, phosphorylation, SUMOylation, and palmitoylation) of *T. cruzi* and *T. brucei*. Proteomic analysis of infective life-cycle stages of T. cruzi has led to the discovery of hundreds of potential CD8 T cell epitopes. The top peptide candidates are currently being tested in vitro and in vivo as T cell-based vaccines for experimental CD. In addition, glycan array analysis of protective antibodies from patients against glycans exclusively expressed by the parasite (as determined by glycomics) has resulted in the discovery and validation of a highly effective carbohydrate-based vaccine for experimental CD. Moreover, we have recently carried out SUMOproteomics, phosphoproteomics, and GPIomics of *T. cruzi* and identified numerous potential molecular targets, which are now being validated. Regarding *T. brucei*, we have recently analyzed the global palmitoylproteome of the parasite and found several potential drug targets, which are currently being validated as well. Systems biology (omics) approaches are becoming increasingly vital molecular tools for the discovery and validation of drug and vaccine targets in kinetoplastids. Considering that protozoan parasites and fungi share many common traits regarding their metabolism and pathophysiological mechanisms, here we will discuss how we can exploit these approaches for the discovery of new therapeutic targets and interventions against pathogenic fungi, including *Paracoccidioides brasiliensis*. Support: NIH grants 1R01AI070655, 3R01AI070655-04S1, 2SO6GM00812, 2G12RR008124-16A1, and 2G12RR008124-16A1S1.

Keywords: Systems Biology, Omics, Kinetoplastids, Fungi, Drug and vaccine development

ASPERGILLOSIS AND FUSARIOSIS

Author: MARCIO NUCCI [1]
Institution: 1. UFRJ; Universidade Federal do Rio de Janeiro

Abstract:
Opportunistic mycoses show distinct regional incidence patterns throughout the world and may exhibit different epidemiologic features depending on the geographic region. A prospective cohort study involving 8 brazilian centers was undertaken in order to characterize the epidemiology of invasive fungal infections in patients with acute myeloid leukemia and in hematopoietic stem cell transplant recipeints. Among 969 patients, 132 (14%) developed an invasive fungal infection. Aspergillosis (58%), invasive candidiasis (15%) and fusariosis (14%) were the most frequent mycoses. The incidence of invasive aspergillosis was 28% among patients with acute myeloid leukemia and 3% among hematopoietic stem cell transplant recipients, whereas fusariosis occurred in 3% of acute myeloid leukemia patients and in 2% of transplant recipients. Among hematopoietic stem cell transplpant recipients, the incidence of invasive aspergillosis was 4 times higher in allogeneic than in autologous transplantation. Likewise, invasive fusariosis was more common in allogeneic transplpant recipients (2.3% vs. 0.9%). The clinical presentation of invasive fusariosis was typical, with frequent positive blood cultures and metastatic skin lesions. However, a substantial number of cases had a cotaneous portal of entry, usually as cellulitis in patients with onychomycosis or intertrigus. Deoxycholate amphotericin B was given as primary treatment for invasive aspergillosis in 75% of cases, despite the poor results compared to voriconazole. This may have been due to budgetary restrictions in the 8 public hospitals. On the other hand, voriconazole was the most frqeuent agent used as second line therapy. The knowledge of the epidemiologic characteristics in a certain region is important both locally but also globally, given the expansion of traveling and migration through different regions of the globe.

Keywords: aspergillosis, fusariosis, invasive mycosis, immunosuppressed

Tuesday, 03 May 2011

FORWARD AND REVERSE GENETIC APPROACHES TO VIRULENCE FACTOR DISCOVERY IN *HISTOPLASMA CAPSULATUM*

Author: CHAD RAPPLEYE [1]
Institution: 1. OSU; Ohio State University

Abstract:
The pathogenesis of *Histoplasma capsulatum* is linked to the yeast phase and the factors produced by cells in this state. We have used a proteomics approach to identify the extracellular proteins produced by this pathogenic phase as these factors may affect host macrophages and immune defense systems. The major components identified from *Histoplasma* yeast culture filtrates include cell-wall related glycosidases and enzymes involved in defense against oxidative stress. Approximately one-fourth of the extracellular proteins represent novel factors with unknown functions. Enriched expression of genes encoding extracellular proteins in the pathogenic phase as compared to non-pathogenic mycelia was used to prioritize factors for functional studies. Using an RNAi sentinel system that co-targets the gene of interest and GFP fluorescence of an expressed gfp transgene, we have generated strains lacking individual extracellular factors. Culture filtrates harvested from these strains confirm depletion of the respective factors and these strains are being employed in cultured macrophage and animal models of histoplasmosis to assess their contribution to *Histoplasma* pathogenesis.
We are also employing *Agrobacterium*-mediated transformation to generate random T-DNA insertions in *Histoplasma* with which to identify mutants in virulence-related genes. To isolate mutations in specific genes, we optimized methods to pool insertion mutants and screen them by PCR for disruptions in a targeted gene of interest. Successive subdivision of pools containing the insertion mutant permits isolation of the desired strain. This methodology enables isolation of gene knock-outs without reliance on homologous recombination. For forward genetics, random T-DNA insertion mutants are being screened in cultured phagocytes for yeast strains with reduced virulence. We developed an efficient assay to measure replication of *Histoplasma* yeast in infected macrophages through the cytopathic effects of yeast on phagocytic host cells. This assay has been adapted to a 96-well plate format allowing for higher throughput screens to be performed. With this virulence screen, we have isolated three *Histoplasma* mutants with reduced replication in phagocytes.

Keywords: Histoplasma, mutation, RNAi, secretion, transformation

SILENCING OF THE ALTERNATIVE OXIDASE (AOX) GENE IN *PARACOCCIDIOIDES BRASILIENSIS*: EFFECT ON CELL METABOLISM AND PATHOGENICITY

Author: HERNANDEZ, O. [1,2,5], ARAQUE, P. [3], ALMEIDA, AJ. [4], TAMAYO, D. [2,1], GARCIA, AM. [2], RESTREPO, A. [2], PELAEZ, C. [3], MCEWEN, JG. [2,5]
Institution: 1. UdeA; Instituto de Biologia, Universidad de Antioquia
2. BCM - CIB; Cellular and Molecular Biology Unit
3. GIEM; GIEM, Universidad de Antioquia
4. ICVS; Life and Health Sciences Research Institute
5. UdeA; School of Medicine

Abstract:
Many biological processes in fungi are dependent on molecular oxygen (O2). The use of O2 as final electron receptor in the respiratory chain of the inner mitochondrial membrane (IMM), can lead to the production of reactive oxygen species (ROS), such as superoxide (O2-), hydrogen peroxide (H2O2) and hydroxyl (OH-) radicals. On the other hand, during host-pathogen interaction ROS are produced by immune cells all of the ROS may ultimately alter the bioenergetic status of the cell or affect essential metabolic pathways, thus representing a toxic stimulus that decreases cell survival. In our laboratory we have generated a *P. brasiliensis* PbAOX-antisense RNA (aRNA) strain, with a ≈80% decrease in gene expression. We demonstrated that PbAOX plays a relevant role in the maintenance of extracellular pH during batch culture growth, crucial for sustaining cellular vitality and viability, and that it is important for detoxification of H2O2 induced by exogenous oxidative stress. In addition, we showed that PbAOX is involved in the response to temperature shifts, assists the *P. brasiliensis* morphological transition, particularly during the yeast-to-mycelia (Y-M) germination and mycelia/conidia-to-yeast (M/C-Y) transition. In addition, an increase in PbAOX expression was observed in *P. brasiliensis* wild type (PbWt) conidia and yeast cells after interaction with activated alveolar macrophages, whereas both fungal morphotypes with down-regulation in PbAOX gene expression were significantly affected in their viability after interaction with these activated phagocytes. Additionally, mice infected with conidia from the PbAOX strain showed an increase survival time and lower fungal burden in their lungs at weeks 8 and 24 after intranasal challenge. These data support the hypothesis that AOX is important in fungal defense against ROS produced by immune cells. Our findings point out AOX as an important virulence factor during the initial interaction with host cells in *P. brasiliensis* infection. Acknowledgement: : Colciencias (project No 221334319183), Corporacion para Investigaciones Biologicas and Universidad de Antioquia supported this work. The National Doctoral Program of COLCIENCIAS supported Orville Hernandez.

Keywords: *P. brasiliensis*, Alternative Oxidase, Pathogenicity, aRNA silencing

MECHANISMS OF SYNTHESIS AND DEGRADATION OF CELL WALL ALPHA GLUCAN IN *PARACOCCIDIOIDES BRASILIENSIS*

Author: NINO-VEGA, G.A. [1], CAMACHO, E. [1], VILLALOBOS, H. [1], BARRETO, L. [1], SAN-BLAS, G. [1]
Institution: 1. IVIC; Instituto Venezolano de Investigaciones Científicas

Abstract:
In *Paracoccidioides brasiliensis*, α-1,3-glucan is the major neutral cell wall polysaccharide of the pathogenic yeastlike (Y), organised as a sort of outer capsule, replacing almost entirely the β-1,3-glucan, while absent in the mycelial (M) phase. It has been proposed as virulence factor in *P. brasiliensis*, *Blastomyces dermatitidis* and *Histoplasma capsulatum*. Loss of α-(1,3)-glucan in *H. capsulatum* by RNA interference of the *AGS1* gene, produced attenuation of the ability to kill macrophages and colonize murine lungs, demonstrating a role for α-(1,3)-glucan in virulence (Mol Microbiol. 2004;53(1):153-165). Chemical analyses of *P. brasiliensis* α-1,3-glucan, synthesized by Ags1p, indicated that it is essentially a linear polysaccharide, with < 3% of α-1,4-linked glucose branches, occasionally attached as single units to the α-1,3-backbone. Our group have been researching the different genes possible involved in the synthesis, regulation and hydrolysis of cell wall α-1,3-glucan in *P. brasiliensis*. A gene with high identity with *H. capsulatum* α-1,4-amylase, expressed preferentially in the pathogenic Y phase, was identified, and complemented an *H. capsulatum amy1* mutant, a result that suggest an important role for its product in the synthesis of α-1,3-glucan in *P. brasiliensis*. *In silico* amino acid analysis of the deduced protein led to the identification of all four conserved regions of the α-amylase family, the critical moieties for biological activity and amino acids associated with the specificity to glucosidic α-1,4-linkages. Also, a single gene for an α-1,3-glucanase was identified and its product analysed, showing α-1,3-glucanase activity. In the present work, we summarize our molecular and biochemical data on *P. brasiliensis* α-1,3-glucan, by proposing mechanisms of synthesis, regulation and hydrolysis of the cell wall α-1,3-glucan in this medically important fungus. This work was supported by Research Project 112 from Instituto Venezolano de Investigaciones Científicas.

Keywords: alpha-1,3-glucan, cell wall, alpha glucanase

TRANSCRIPTIONAL ANALYSIS OF *PARACOCCIDIOIDES BRASILIENSIS* IN THE PRESENCE OF ANTIFUNGAL

Author: PEREIRA, M. [1]
Institution: 1. UFG; Universidade Federal de Goiás

Abstract:
Paracoccidioides brasiliensis is a dimorphic and thermo-regulated fungus which is the causative agent of paracoccidioidomycosis (PCM), an endemic disease widespread in Latin America that affects 10 million individuals. The mycosis has also been reported among patients with AIDS. The conventional drugs used for treatment of PCM are sulfonamides, amphotericin B and imidazole derivatives such as ketoconazole, itraconazole and fluconazole. The mechanism of action of these drugs has been investigated. However, no studies were also carried out for *P. brasiliensis* yet. Antifungal agents exert their activity through a variety of mechanisms, some of which are poorly understood. Novel approaches to characterize the mechanism of action of antifungal agents will be of great use in the antifungal drug development process. The aim of the current study was the identification of up and down regulated genes from *P. brasiliensis* after 1 hour and 2 hours of exposition to itraconazole and sulfametoxazole. By analyzing transcriptional profile and the validation of the genes, we identified genes described as regulated in other microorganisms in the presence of these drugs. However, other new genes were identified. To sulfametoxazole, several transcripts related to mitochondrial function were down regulated. Sulfa drugs act by competitive inhibition of the enzyme dihydropteroate synthase, a key enzyme involved in folate synthesis, which is required for mitochondrial protein synthesis. Triazole drugs act by blocking the ergosterol, an essential cell membrane component, biosynthetic pathway through binding to and inhibition of the lanosterol 14-α demethylase enzyme, encoded by the erg11. In this study, most of the regulated genes were involved in lipid metabolism including the precursors of ergosterol. The genes erg11, erg6, erg5 and erg3 were up regulated. In addition, were found genes involved in cell stress response, drug efflux, small molecule transport, elongation and transcription factors, cell wall and membrane, and hypothetic proteins, which had not yet been identified as responsive to these drugs. These data shed light on the mechanism of action of these classes of antifungal agents and demonstrate the potential utility of gene expression profiling in antifungal drug development. Financial Support: CNPq, FINEP.

Keywords: Antifungal, Drugs, *Paracoccidioides brasiliensis*, Transcriptional analysis

INFLUENCE OF 17B-ESTRADIOL ON GENE EXPRESSION OF PARACOCCIDIOIDES BRASILIENSIS DURING MYCELIA TO YEAST TRANSITION

Author: Jata Shankar [1,2,3], Karl V. Clemons [1,2,3], Laurence F. Mirels [1,2,3], Jomar P. Monteiro [1,2,3], Thomas D. Wu [4], David A. Stevens [1,2,3]
Institution: [1] CIMR - California Institute for Medical Research (San Jose, CA), [2] SCVMC - Santa Clara Valley Medical Center (San Jose, CA 95128), [3] SU - Stanford University (Stanford, CA), [4] GI - Genentech, Inc. (South San Francisco, CA)

Abstract:
Infection by *Paracoccidioides brasiliensis* is initiated by inhalation of conidia or mycelial (M) fragments by the host, which subsequently differentiate into yeast (Y). Epidemiological studies indicate a predominance of paracoccidioidomycosis in adult men compared to women. Furthermore, in vitro and in vivo studies suggest that the female hormone (17β-estradiol, E2) plays an important role in regulating morphological transition, inhibiting M to Y transition. In our current studies we have examined the molecular mechanism of how E2 inhibits M to Y transition. We assessed temporal gene expression in *P. brasiliensis* (Pb01) by evaluating transcript levels in the presence or absence of E2 at various time points through 9 days of the M to Y transition. We employed an 11,000 element random-shear genomic DNA microarray previously developed in our laboratory and verified the results using RT-PCR. Overall, the abundance of transcripts exhibited a general decline during the M to Y transition in both controls and E2-treated cultures. In comparison with controls, E2 treatment altered transcript levels by at least twofold in genes represented by 550 array elements, with 331 showing up-regulation and 219 showing down-regulation at one or more time points. We sequenced these clones to identify the underlying genes and biological function. Genes with lower initial expression after exposure to E2 belonged to pathways involved in heat shock response (hsp-70), energy metabolism, and several retrotransposons. The Y-specific genes mannosyltransferase and Y20 demonstrated lower, delayed expression in E2-treated cultures. Hydrophobin, an M-specific gene, maintained higher expression in the presence of E2 relative to controls. A number of genes potentially involved in signaling, such as palmitoyltransferase (erf2), RhoA GTPase activating protein, phosphatidylinositol-4-kinase, and a predicted serine/threonine-protein kinase were down-regulated by E2. One exception was an arrestin domain-containing protein, which showed increased expression. In addition, genes related to ubiquitin-mediated protein degradation, and oxidative metabolism genes were up-regulated by E2. This study showed that E2 delays or blocks expression of some transcripts that are potentially involved in M to Y transition and continued analysis of these data is ongoing.

Keywords: Paracoccidioides, form transition, estradiol, gene expression, microarray

POST-GENOMICS AND ANTIFUNGAL DEVELOPMENT

Author: MARIA SUELI FELIPE [1]
Institution: 1. UnB; Universidade de Brasilia

Abstract:
The prevalence of invasive fungal infections (IFIs) has increased steadily worldwide in the last few decades. IFIs have historically been associated with high morbidity and mortality, partly because of the limitations of available antifungal therapies, including side effects, toxicities, drug interactions and antifungal resistance. The search for alternative therapies and/or the development of more specific drugs is a challenge that needs to be met. In silico analyses and manual mining selected initially 57 potential drug targets, based on 55 genes experimentally confirmed as essential for C. albicans or A. fumigatus and other 2 genes (kre2 and erg6) relevant for fungal survival within the host. Orthologs for those targets were also identified in eight human fungal pathogens (*C. albicans*, *A. fumigatus*, *B. dermatitidis*, *P. brasiliensis*, *P. lutzii*, *C. immitis*, *C. neoformans* and *H. capsulatum*). Of those, 10 genes were present in all pathogenic fungi analyzed and absent in the human genome. We focused on four candidates: trr1 that encodes for thioredoxin reductase, rim8 encoding for a protein involved in the proteolytic activation of a transcriptional factor in response to alkaline pH, kre2 that encodes for α-1,2-mannosyltransferase and erg6 that encodes for Δ(24)-sterol C-methyltransferase. Of the four selected potential targets obtained, only Trr1 and Kre2 showed a reasonable sequence identity to the templates found in PDB. Also, we performed the homology modeling to predict 3D protein models only for these two proteins. To investigate the stability of Trr1 and Kre2 models, molecular dynamics simulations were performed and revealed that the evolution of the systems is very stable. With the models stable, virtual screening for select the main small molecules that interact with them was performed. A bank of commercially available compounds was docked with the models by virtual screening simulations. Small molecules that interact with the models were ranked and, among the best hits, 37 and 20 molecules were finally selected as putative inhibitors of Kre2 and Trr1, respectively. Our data show that the comparative genomics analysis of eight fungal pathogens enabled the identification of new potential drug targets conserved among fungi and absent in the human genome. Also, virtual screening of combinatorial libraries offered new perspectives on technological development and innovation of antifungal agents against human pathogens. Funding-FAP/DF,CNPq

Keywords: comparative genomics, post-genomic analysis, antifungal development

LESSONS FROM PATIENTS WITH PARACOCCIDIOIDOMYCOSIS: THE HOST-FUNGUS INTERACTION STILL OUT OF (OUR) CONTROL?

Author: Gil Benard [1]
Institution: [1] FMUSP - Laboratory of Dermatology and Immunodeficiencies (Tropical Medicine Institute, University of São Paulo - Rua Doutor Costa Júnior)

Abstract:
In the last years basic and clinical immunological research has provided us with significant new data on the host-parasite interaction in paracoccidioidomycsis, which, however, has not yet translated into direct benefits to patient management. We still don't know what makes some few among hundreds or thousands of exposed individuals to be susceptible of developing the acute/subacute form of the disease, nor what mechanisms underly the development of the chronic form of the disease in individuals who have long been infected (sometimes decades ealier). Fungus virulence factors are also not well known. The evidence that gp43 would represent a virulence factor may be challenged by the fact that it is mutated in the new P. lutzii species, which causes a severe disease otherwise similar to that one caused by P. brasiliensis. We describe here some patients who presented clinical conditions that exemplify the difficulties in understanding the host-parasite relationship in PCM. The first patient is a young man who concomitantly presented oral and facial lesions which, on immunohistochemistry, showed distinct immune responses patterns: Th-2/Th-17 and Th-1, respectively. We also present two children with the severe, acute form of the disease, in whom the clinical manifestations could, paradoxically, only be controlled by immunosuppressive doses of corticosteroids. We also present two additional cases in which the immune responses apparently became uncontrollable and led to fatal inflammatory syndromes such as the adult respiratory distress syndrome and septic shock. The elucidation of immunological mechanisms underlying these atypical cases may help to understand better the immunopathogenesis of PCM.
Financial support: Fapesp and CNPq

Keywords: Patient, P. brasiliensis, P. lutzii, Lesion

THE CLINICAL AND EPIDEMIOLOGICAL FEATURES OF PARACOCCIDIOIDOMYCOSIS IN THE SOUTHEAST OF BRAZIL: REPORT OF A COHORT OF 1 000 PATIENTS.

Author: Roberto Martinez [1]
Institution: [1] FMRP-USP - Fac Medicina de Ribeirão Preto-USP (Av Bandeirantes,3900-14048-900 Ribeirão Preto-SP)

Abstract:
Introduction - The information about the epidemiology and clinical features of paracoccidioidomycosis mainly results from the observation of large case series. This study reports original data about 1 000 cases from the Ribeirão Preto region, Northeast of the State of São Paulo, Brazil. Methodology - This is a retrospective study of patients diagnosed and treated for paracoccidioidomycosis between 1970 and 1999 at the University Hospital, FMRP-USP. The incidence of the disease was estimated in relation to the population of various municipalities in the district of Ribeirão Preto. Results - Between 1980 and 1999 the mean incidence of paracoccidioidomycosis was 2.70 cases/100,000 inhabitants/year. The patients ranged in age from 3 to 85 years old (mean = 40.8) and the male:female ratio was 6:1. About 94% of the patients had resided or worked in a rural area. Excessive intake of distilled drinks and smoking habit were reported by 37% and 65% of the patients, respectively. Other infectious and parasitic diseases, concomitant or not with paracoccidioidomycosis, were Chagas' disease, tuberculosis, HIV/AIDS, leishmaniasis, leprosy, and strongyloidiasis. The acute/subacute form of paracoccidioidomycosis was detected in 25% of the cases, predominating among patients aged 3 to 30 years old , among women, and among Blacks. The chronic form was the most common presentation in patients older than 30 years, in men and in patients with a lighter skin. Sulfamide or azole drugs were used to treat most of the patients, 85% of whom were cured or experienced clinical improvement. Recurrence and death attributed to paracoccidioidomycosis were observed in 10% and 8% of cases, respectively. Conclusion- Evidence of hyperendemicity of paracoccidioidomycosis was detected in the geographic area of Ribeirão Preto, SP, Brazil. There was a high proportion of cases with the acute/subacute form, with an association existing between clinical form and age, gender and ethnic group.

Keywords: Paracoccidiomycosis, Epidemiology, Incidence, Sulfamide drugs, Azole drugs

THE LUNG IN TREATED PARACOCCIDIOIDOMYCOSIS PATIENTS: NEW INSIGHTS INTO AN OLD PROBLEM.

Author: AN COSTA [1], ALPALBUQUERQUE [1], ASKMAGRI [2], G BENARD [3], RAKAIRALLA [1], M SHICANAI-YASUDA [2], CRRCARVALHO [1]
Institution: 1. HC-FMUSP; Pulmonary Division, Heart Institute (InCor), USP
2. HC-FMUSP; Infectious Diseases Division, Hospital das Clínicas, USP
3. HC-FMUSP; Laboratory of Dermatology and Immunodeficiencies

Abstract:
RATIONALE: Paracoccidioidomycosis (PCM) is the most frequent systemic mycosis in South America, and 80% of world cases occur in Brazil. Pulmonary involvement is the main feature in chronic form of disease, with some patients developing residual respiratory abnormalities after treatment. In this study we evaluated lung tomographic and functional abnormalities in PCM patients. METHODS: Prospective evaluation of 49 post-treatment lung PCM patients with chest high-resolution computed tomography (HRCT), spirometry, plethysmographic, cardiopulmonary exercise test, six minute walk test (6MWT) and respiratory quality of life questionnaire. RESULTS: Mean age was 57±7 years. Forty-nine patients were current or former smokers, mean length of 38.5±1.4 pack-years. Mean time elapsed between treatment institution and enrollment was 6±3.9 years, and mean treatment lengh was four years. Mean initial serology was 106±42 and mean final serology was 5±1. Main HRCT findings were architecture distortion (92%), septal thickening and reticular opacities (90%), bronchial wall thickening (84%), peribronchovascular interstitial thickening and nodules (64%) and ground glass opacities (48%). Air trapping was found in 78% of patients. The radiologic findings were diffusely present in all lung zones in 84% of patients. Pulmonary function tests showed: forced vital capacity 3.8±0.9L (93±18% predicted), forced expiratory volume in 1 second 2.5±0.7 L (79.6±20%), total lung capacity 6.2±1.3 L (101±16%), residual volume 2.5±0.9 L (125 ± 40%), and DLCO 72%. Cardiopulmonary exercise test showed: maximum oxygen uptake 21.0±1,2 mL/kg/min (66±17%), maximal work rate 73,0±9,2%, maximal heart rate 73,5±9,1%, oxygen pulse 10.9±2,8 mL/beat, minute ventilation 191.7±22.2 L/min (53.7±8,5%) and minute ventilation/maximal voluntary ventilation 0.61 ± 0.09, with a mean ventilatory reserve of 39%. Mean walked distance (6MWT) was 574±21 meters (85% of predicted). Saint George Respiratory Questionnaire mean values were 607±78 points, showing low impact of the pulmonary symptoms in quality of life. CONCLUSIONS: Herein we indicate, in disagreement with current literature, that adequately treated PCM patients present very frequent radiological abnormalities not solely attributed to tobacco exposure. However, these findings, associated to mild static and dynamic functional abnormalities and with slight abnormal exercise capacity, are not linked to a severe life impact.

Keywords: LUNG, respiratory sequelae, treated paracocicdioidomycosis

LIVER PARACOCCIDIOIDOMYCOSIS IN CHILDREN

Author: RICARDO PEREIRA [1]
Institution: 1. FCM UNICAMP; Faculdade de Ciencias Medicas UNICAMP

Abstract:
The Paracoccidioidmycosis is rare in pediatrics with few descriptions of the clinical presentation and especially the hepatic involvement. The objective of this paper was to present 41 cases of liver paracoccidioiomycosis in patients younger than 16 years and compare the clinical presentation, laboratory and evolution in Pediatric Patients with paracoccidioidomycosis without hepatic involvement. We observed that patients with liver involvement have significantly lower age, higher number of male patients and a higher proportion of undernourished. Clinical manifestations of weight loss, malaise, anorexia, pallor, and splenomegaly were more frequent among patients with liver involvement. When comparing the results of the complete blood count in relation to liver involvement there was a lower hemoglobin concentration, lower lymphocyte count and platelet counts with statistical difference. In relation to serum protein electrophoresis to compare the groups showed, with statistical difference, lower albumin concentration. The results of multivariate logistic regression shows a combined influence of albumin on admission (every 1 unit of albumin on admission the risk of liver involvement decreases 81.8%, or every reduction of 1 unit albumin on admission the risk of liver involvement increases 5.5 times (OR = 5.49) and age (every 1 year of age the risk of liver involvement decreases 23.5%, or every reduction of 1 year of age the risk of liver involvement increases 30.7% (OR = 1.31). The results of ROC curve analysis for laboratory tests on admission, using the liver involvement as the gold standard, show that it is possible to establish cutoff levels from which the liver involvement is more likely for the variables albumin (≤ 3.05 g / dL), hemoglobin (≤ 9.2 g / dL) and lymphocytes (≤ 2508/mm3). This study showed that it is possible to predict the hepatic involvement by *Paracoccidioides brasiliensis* using clinical data (age) and laboratory (albumin, hemoglobin and lymphocytes).

Keywords: liver, paracoccidioidomycosis, albumin , hemoglobin, children

CHEMOKINE REGULATION OF IMMUNITY TO HISTOPLASMA CAPSULATUM

Author: GEORGE DEEPE [1]
Institution: 1. IC; University of Cincinnati Col. of Med.

Abstract:
Resolution of infection with the pathogenic fungus, Histoplasma capsulatum, requires collaboration among numerous constituents of the host immune response. Although the necessity for particular cytokines has been established, we have examined the contribution of chemokines and their receptors on host defenses to this fungus. Mice lacking the chemokine receptor, CCR2, succumb to an otherwise sublethal infection. The failed immune response is caused by elevated levels of interleukin-4. The absence of CCL7 and CCL2, two ligands for CCR2, is responsible for altered production of interleukin-4. Protective immunity is restored by neutralizing interleukin-4 or by adoptive transfer of antigen-primed dendritic cells plus elimination of CD4+ cells prior to, but not at the time of, infection. Hence, CD4+ cells are deleterious to host defenses in this model. We have also explored how IL-4 modulates host response. In a series of in vitro studies, we demonstrated that activation of macrophages by the cytokine granulocyte-macrophage colony stimulating factor (GN-CSF) inhibits intracellular growth of the fungus by depriving reducing zinc content in both the macrophage and the fungus. Treatment of macrophages exposed to GM-CSF with IL-4 restores intracellular growth in association with an increase in zinc. In contrast to the aove, the absence of the chemokine receptor CCR5 accelerates clearance of infection. The improved outcome is a result of a shift in the balance between regulatory T cells and interleukin-17+ CD4+ T cells. The lack of CCR5, or one of its ligands, CCL4, dampens migration of regulatory T cells from the thymus and inhibits their expansion in the lungs, but facilitates Th17 cell expansion. The absence of CCR5 also mitigates the deleterious effects of treatment with anti-tumor necrosis factor-α antibody. These findings highlight the complex nature of chemokine signaling and the interaction between chemokines and cytokines in this infectious disease.

Keywords: Chemokine, Fungal, Lung, Rodent, Immunity

N-GLYCANS FROM TLR2 HETERODIMERS AS TARGETS FOR A LECTIN THAT CONFERS PROTECTION AGAINST PARACOCCIDIODOMYCOSIS

Author: Maria Cristina Roque Barreira [1]
Institution: [1] FMRP- USP - Faculdade de Medicina de Ribeirão Preto- USP (Av. Bandeirantes 3900- 14049900-Ribeirão Preto- SP)

Abstract:
ArtinM, a D-mannose binding lectin, confers resistance to experimental infections with *Paracoccidioides brasiliensis*. ArtinM-treated mice, in comparison to the non-treated ones, show lower fungal burden in their organs, higher production of IL-12, IFN-gamma and TNF-alpha. The host advantageous effects provided by ArtinM are not verified in IL-12 KO mice. Murine macrophages stimulated with ArtinM produce high IL-12 levels, a response that is not verified when the cells were derived from MyD88 KO mice or in the presence of D-mannose. The role of Toll-like receptors in the macrophage response was delineated in ArtinM-stimulated macrophages from TLR2- and TLR4-deficient mice; the IL-12 production was blocked only in the cells from TLR-2 KO mice. The facts that the TLR2 ectodomain has 4 N-glycans and that ArtinM recognizes specifically the trimannoside core of N-glycans explain the lectin interaction with the PRR. The ArtinM stimulus of HEK 293 cells transfected with plasmids coupled with the TLR-2 ectodomain and a NF-kB-luciferase reporter gene promoted NFkB translocation, which was not verified when ArtinM was pre-incubated with the D-mannose or was used to stimulate TLR4 transfected cells. To better understand the mechanisms responsible for the ArtinM immunomodulatory effect in PCM, the HEK293 cells were also transfected with TLR2 heterodimers (TLR2/1 and TLR2/6), as well as with coreceptors/ accessory molecules (CD36, CD14, MD-2). The ArtinM stimulus promoted NF-kB activation in cells expressing TLR2/1 and TLR2/6. No activation was determined by similar AM concentrations in cells expressing TLR4. The co-receptor CD36 was shown to be especially important in the process, because in its absence concentrations 10 times higher of ArtinM were required to induce NF-kB activation. The exact role exerted by the scavenger glycosylated receptor CD36 on the signaling triggered by ArtinM is under investigation.

Keywords: immunomodulation, Toll-like receptor, N-glycan, lectin, Th1 immunity

VIABILITY OF A THERAPEUTIC VACCINE IN PARACOCCIDIOIDOMYCOSIS. AN UPDATE

Author: TABORDA, C.P. [1,2], TRAVASSOS, L.R. [3]
Institution: 1. USP; Departament of Microbiology - ICB/USP
2. IMT/LIM-53-USP; Laboratory of Medical Mycology IMT/SP - LIM 53
3. UNIFESP; Department of Microbiology, Immunology and Parasitology

Abstract:
Treatment of paracoccidiodomycosis (PCM) patients with antifungal drugs is the required procedure, although there is no assurance of complete cure after prolonged periods of drug administration and relapsing disease is a common event. Attempts at immunotherapy have concentrated on the gp43 and derived peptide 10 which carries a promicuous T-CD4+ epitope. Most of the successful protection experiments using P10 were carried out in BALB/c mice using complete Freundâ€™s adjuvant. Recently, alternative ways of delivering P10 using different adjuvants such as alumen and a cationic lipid, as well as a plasmid minigene encoding P10, Salmonella enterica FliCd flagellin and P10, nanoparticles and dendritic cells pulsed with P10. The following immunization procedures were used in both prophylactic and therapeutic protocols: (A) Immunization using different adjuvants rendered the most promising results using the cationic lipid, that led to a significant reduction in the fungal burden of lungs from intratracheally infected normal and anergic animals; (B) Immunization of P10 minigene in plasmid DNA accompanied or not by IL-12 gene therapy led to significant reduction of fungal burden in the lung, spleen and liver. Increased production of IL-12 and IFN-γ with low IL-4 in lung homogenates was obtained. The DNA-based vaccine encoding P10 also generated cells with regulatory and memory phenotypes; (C) The peptide was genetically fused to the central region of flagellin FliCd or was just admixed with free flagellin and nasally administered into BALB/c mice. The treatment of mixed flagellin and P10 promoted higher protection than the fused flagellin-P10 preparation; (C) The delivery of P10, encapsulated within PLGA-DMSA polymeric blends (nanoparticles), was used associated with sulfamethoxazole and the animals had a significant decrease in the fungal burden of lungs; (D) Dendritic cells pulsed with P10 were transferred to BALB/c mice previously infected with *P. brasiliensis* rendering a significant reduction in the lung fungal burden. With the encouraging results obtained using different preparations of P10, the next step should be pursued in pre-clinical models aiming at toxicity and pharmacokinetics studies to select the most effective formulation for clinical trials.

Keywords: *Paracoccidioides*, vaccine, P10, gp43

IMMUNE RESPONSE AND HISTOPATHOLOGICAL ASPECTS OF PULMONARY FIBROSIS IN EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS

Author: CANO, L.E [1,2,3], LOPERA, D [1], NARANJO, T.W [1,3], GONZALEZ, A [1,2], RESTREPO, A [1], LANZI, H.L [4]
Institution: 1. CIB; Corporacion para Investigaciones Biologicas
2. UdeA; Universidad de Antioquia
3. UPB; Universidad Pontificia Bolivarina
4. Fiocruz; Fundacao Oswaldo Cruz

Abstract:
Paracoccidioidomycosis (PCM is classified into 4 clinical groups: asymptomatic or subclinical, juvenile acute/subacute, adult or chronic and residual forms. The chronic presentation is the most common (90%) of all human cases and takes months to years to become apparent. At diagnosis, pulmonary lesions are frequent with lesions being at different stages of development including fibrosis, a sequel that may also occurs de novo during antifungal therapy. We have studied certain immune, histological and radiological aspects of the pulmonary lesions by using our experimental pulmonary PCM model (male BALB/c mice intranasally inoculated with *Paracoccidioides brasiliensis (Pb)* conidia). Development of lesions was follow-up by histopathology (early and chronic periods) and high-resolution computed tomography-HRCT (chronic periods). The local immune response was measured by determining different cytokines in supernatants of pulmonary homogenates. The results showed that during the early stages of infection (2hs to 2 wks) the lungs of Pb-infected mice presented a bronchopneumonic acute type response with PMNs accumulation that fused with each other to constitute a large, ill-defined accumulation located preferentially at the peribronchiolar level that evolved towards lympho-histo-plasmocytic infiltrates. This period of infection was accompanied by an intense pro-inflammatory cytokine burst. During the chronic infection stages granulomatous reaction arranges in 3 different lesion patterns: nodular-diffuse, confluent and pseudo-tumoral that were mainly located around the hilus and affected more frequently the left lung. At the 8th and 12th wks post-challenge, collagenesis reached its highest peak with particular involvement of the periarterial space. Cytokine profiles changed showing an immunosuppressive pattern. We also evaluated the effects of itraconazole (ITC) treatment, alone or plus pentoxifilline (PTX) beginning at 4th or 8th week after *Pb*-infection. The ITC+PTX therapy resulted in a significantly and more rapid reduction of granulomatous inflammation and pulmonary fibrosis when compared with the results of classical antifungal therapy using ITC alone. These promissory results open a new window for implementing novel treatment strategies for PCM patients and with other diseases leading to fibrotic sequelae.

Keywords: Paracoccidioidomycosis, Immune response , Histopathology, Fibrosis

Wednesday, 04 May 2011

MODIFYING THE INTRACELLULAR FATE OF *HISTOPLASMA CAPSULATUM*

Author: NOSANCHUK [1]
Institution: 1. Einstein; Albert Einstein College of Medicine

Abstract:
Histoplasma capsulatum variety *capsulatum* is the most prevalent thermally dimorphic endemic mycoses in North America and a major cause of disease in Central and South America. Current antifungal drugs are powerful therapeutics, but excess mortality continues to occur in immunocompetent and immunocompromised patients with histoplasmosis. Monoclonal antibodies are powerful tools for studying diverse aspects of the biology of pathogenic microbes. We have shown that antibodies to cell surface antigens of *Histoplasma capsulatum*, including heat shock protein 60 (Hsp60), histone 2B, and the M antigen, can modify the pathogenesis of histoplasmosis. Exploration of the mechanisms involved in the altered host-pathogen interactions in the presence of antibody have illuminated interesting aspects of pathogenesis, including new findings regarding phagocytic processes, intracellular survival and T cell stimulation. Interestingly, these studies can also inform us about basic antibody function, such as the differential impact of antibody isotype on disease outcome. Furthermore, we have recently utilized *H. capsulatum*-binding antibodies to study physical interactions, such as fungal cell aggregation and its influence on pathogenesis. The antibodies have also facilitated the study of the recently described secreted fungal vesicles and have opened avenues for exploring protein-protein interactions that have thus far allowed us to gain new insights into the regulation and trafficking of key cellular proteins. Hence, monoclonal antibodies are an extraordinary component of our toolbox for the exploration of *H. capsulatum*. These antibodies also are a platform for therapeutic development.

Keywords: *Histoplasma*, Antibody, Pathogenesis

THE USE OF PROTEOMICS TO STUDY HOST-*PARACOCCIDIOIDES BRASILIENSIS* INTERACTIONS

Author: SOARES CMA [1]
Institution: 1. UFG; Universidade Federal de Goias

Abstract:
The use of proteomics to study host-*Paracoccidioides brasiliensis* interactions Celia Maria de Almeida Soares, Laboratorio de Biologia Molecular, Instituto de Ciencias Biologicas, Universidade Federal de Goias, Goiania, Goias, Brazil. email:cmasoares@gmail.com *Paracoccidioides brasiliensis* is a dimorphic fungal pathogen that causes pulmonary and systemic disease in humans. Two-dimensional electrophoresis in combination with mass spectrometry has become a powerful tool for studying the proteome of a number of pathogens. For fungi such as *P. brasiliensis* such information is nascent. We have addressed an important aspect of the *P. brasiliensis* interplay with the host: the influence of iron availability on the fungal metabolic processes. Iron acquisition is critical to cellular function and survival. During infection of mammals, the host limits the access of iron to microbial pathogens by a variety of means. We demonstrated an increased fungal burden in mice treated with iron supplement, indicating that iron availability promotes an increased susceptibility of mice to *P. brasiliensis* infection. The proteome of *P. brasiliensis* during iron starvation revealed a remodeling in energy metabolism increasing glycolytic activity , compensating the decrease of aerobic pathways that are mostly iron dependent. Putative virulence factors are induced upon iron deficiency. Genes induced by iron deficiency are also regulated in fungal cells during infection. Regulation of iron uptake and iron source preference has been investigated by our group. Both the reductive and siderophore iron uptake systems are found in *P. brasiliensis*. Upon iron deficiency it was observed the secretion of hydroxamate siderophores; reverse-phase HPLC analysis revealed dimerum acid in this fungi. *P. brasiliensis* can grow in the presence of hemoglobin as iron source; a cell surface protein, member of a putative hemoglobin-receptor gene family, was induced in the presence of hemoglobin and had been characterized as a GPI anchored protein. Overall our results indicate that *P. brasiliensis* utilizes iron sources within the host and requires reductive and non-reductive iron uptake systems. Proteomic analysis of yeast cells in the presence of hemoglobin is under progress. Financial support: FINEP, CNPq, FAPEG.
Keywords: *Paracoccidioides brasiliensis*, iron metabolism, proteome, siderophores, iron sources

COMPARATIVE GENOMIC STUDIES OF EMMONSIA SPP., *PARACOCCIDIOIDES BRASILIENSIS* AND OTHER ONYGENALES

Author: CLAY, O.K. [1,2], WHISTON, E. [3], GALLO, J.E. [1], MUNOZ, J.F. [1,4], MISAS, E. [1,4], TAYLOR, J.W. [3], MCEWEN, J.G. [1,4]
Institution: 1. CIB; Corporacion para Investigaciones Biologicas
2. URosario; Universidad del Rosario
3. UC Berkeley; University of California, Berkeley
4. UdeA; Universidad de Antioquia

Abstract:
Several of the human-pathogenic dimorphic fungi belong to the order Onygenales. Nine genera from this order (*Paraccoccidioides*, *Histoplasma*, *Blastomyces*, *Coccidioides*, *Uncinocarpus*, *Arthroderma*, *Trichophyton*, *Microsporum* and *Ascosphaera*) are already represented by genomes of one or more isolates that have been sequenced, assembled and/or annotated. All known *Paracoccidioides* isolates belong to an inner clade that includes the pathogenic genera *Blastomyces* and *Histoplasma* as well as the typically non-pathogenic genus *Emmonsia*. As no *Emmonsia* genome sequences were available, we sequenced the genomes of *Emmonsia parva* UAMH 139 and *Emmonsia crescens* UAMH 3008 using Illumina paired-end, 101-bp read technology (one insert size) and are currently assembling, annotating, and analyzing them. Our initial de novo assembled scaffolds of the two *Emmonsia* species indicate that *E. crescens* UAMH 3008 and especially *E. parva* UAMH 139 are closer to *Blastomyces dermatitidis* than to other fully sequenced fungal species, in agreement with some earlier studies based on single genes. Thus, the coverage, syntenic regions and proximity should allow direct reference annotation of the *E. parva* protein-coding genes that are also present in *B. dermatitidis*. In the interest of identifying and understanding virulence factors and phase transitions in *Paracoccodioides brasiliensis*, our ultimate task is to compare the *E. parva* and *E. crescens* sequences to other Onygenales genomes within and beyond the inner clade, in a search for functionally relevant differences between the essentially non-pathogenic *Emmonsia* genomes and *P. brasiliensis* or other closely related pathogen genomes. In this presentation we discuss first results based on our initial working scaffolds, direct short read analyses, and ortholog alignments for selected genes.

Keywords: comparative genomics, dimorphic fungi, Onygenales, bioinformatics, short read sequencing

DOES *PARACOCCIDIOIDES BRASILIENSIS* USE EXTRACELLULAR VESICLES TO COMMUNICATE WITH THE HOST?

Author: PUCCIA R. [1]
Institution: 1. UNIFESP-EPM; Universidade Federal de São Paulo

Abstract:
We have characterized extracellular vesicles from *Paracoccidioides brasiliensis*. We used isolate Pb18 (main species S1) as a model, in comparison with Pb3 (phylogenetic group PS2). We showed that *P. brasiliensis* antigens were present in vesicle preparations from both isolates, as observed in immunoblots revealed with sera from PCM patients. We demonstrated that vesicles carry highly immunogenic α-linked galactopyranosyl (α-Gal) epitopes. TEM images revealed immunogold labeling with Marasmius oreades agglutinin (MOA), which recognizes terminal α-Gal, on the surface and in the lumen of Pb18 vesicles; anti-α-Gal abundantly labeled the cell wall and inside vacuoles. We characterized the secretome of Pb18 using tandem mass spectrometry. We found 123 proteins highly enriched or exclusively identified in vesicles, against 200 in vesicle-free fractions and 105 detected in both fractions at different percentages of abundance relative to total secretome. Vesicle proteins showed diverse functions, but groups containing signaling proteins, GTPase-mediated signal transduction, cell division, and nucleosome organization were highly enriched in vesicles relative to whole genome. Interacting network detected 45 Pb18 vesicles proteins with enriched biological process GO categories. Vesicle lipids were fractionated in silica-gel 60 and analyzed. In Pb18 vesicles, two different species of phosphatidylinositol, namely alkyl-C16:0-acyl-C18:1 and alkyl-C16:0-acyl-C18:2, were absent in Pb3. The predominant fatty acid in Pb3 vesicles was oleic acid (C18:1) followed by linoleic acid (C18:2), which was the most abundant in Pb18 vesicles. The prevalent sterol in Pb3 and Pb18 vesicles was ergosta-7,22-dien-3βol, at a ratio of 8 in Pb3 and 1.13 in Pb18. Extracellular vesicles from Pb3 and Pb18 were both able to stimulate cytokine production in RAW264.7 macrophages. However, tumor necrosis factor-α (TNF-α) production was higher when cells were stimulated with vesicles isolated from Pb3, while the production of interleukin-10 was higher when macrophages were stimulated with Pb18 vesicles. Thus, *P. brasiliensis* vesicles carry immunomodulatory components that might differentially interfere with the host-fungal relationship. **Support: FAPESP, CNPq, NIH (grants # 5G12RR008124-16A1 and 5G12RR008124-16A1S1)**

Keywords: vesicles, secretome, lipidome