THEMATIC PROJECTS

FAPESP
THE STATE OF SÃO PAULO
RESEARCH FOUNDATION

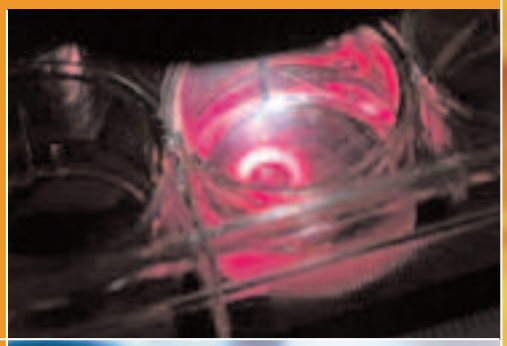
OPPORTUNITIES FOR HEALTH RESEARCH IN BRAZIL

GENETICS

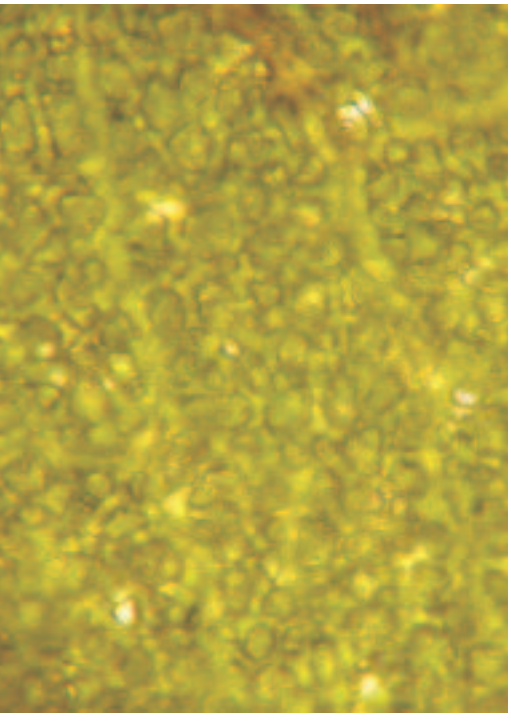
MICROBIOLOGY

IMMUNOLOGY

PARASITOLOGY



EXPLORATORY RESEARCH RECOGNIZED WORLDWIDE



Cancer, genetics, immunology, biochemistry, tropical diseases, medicine. In these and many other sub-areas of Health science, Brazilian scientistas contributed results recognized worldwide.

FAPESP, the State of São Paulo Research Foundation, is one of the main Brazilian agencies for the promotion of research. The foundation supports the training of human resources and the consolidation and expansion of research in the state of São Paulo.

Thematic Projects are research projects that aim at world class results, usually gathering multidisciplinary teams around a major theme. Because of their exploratory nature, the projects can have a duration of up to four years.

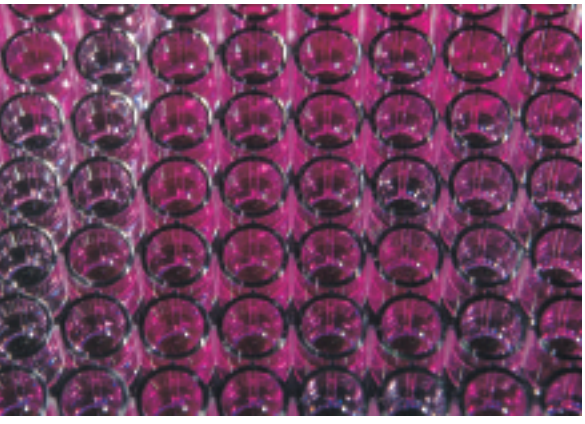
SCIENTIFIC OPPORTUNITIES IN SÃO PAULO, BRAZIL

Brazil is one of the four main emerging nations. More than ten thousand doctorate level scientists are formed yearly and the country ranks 15th in the number of scientific papers published.

The State of São Paulo, with 40 million people and 34% of Brazil's GNP responds for 53% of the science created in Brazil. The state hosts the University of São Paulo (USP) and the State University of Campinas (Unicamp) , both classified among the 200 best in the world by the Times Higher Education Suplement (THES), the growing University of The State of São Paulo (UNESP), Federal University of ABC (ABC is a metropolitan region in São Paulo), Federal University of São Carlos, the Aeronautics Technology Institute (ITA) and the National Space Research Institute (INPE).

Universities in the state of São Paulo have strong graduate programs: the University of São Paulo forms two thousand doctorates every year, the State University of Campinas forms eight hundred and the University of the State of São Paulo six hundred.

In addition to the three state universities the state has 19 research institutes, three federal universities of international research level and most of Brazilian industrial R&D. The state houses more than 10 thousand fulltime faculty and 130 thousand students. São Paulo alone, produces more scientific papers than any country in Latin America, except for Brazil.



FAPESP: SUPPORT FOR RESEARCH IN SÃO PAULO

The State of São Paulo Research Foundation (FAPESP) promotes scientific research in the State of São Paulo, Brazil. Through a robust program of fellowships and research grants it supports fundamental and applied research.

Created in 1962, the foundation is entitled by the State Constitution to 1 per cent of the tax revenues of the state of São Paulo. FAPESP has a sizable endowment and has already supported, over these 46 years, 89,000 fellowships and 80,000 research awards.

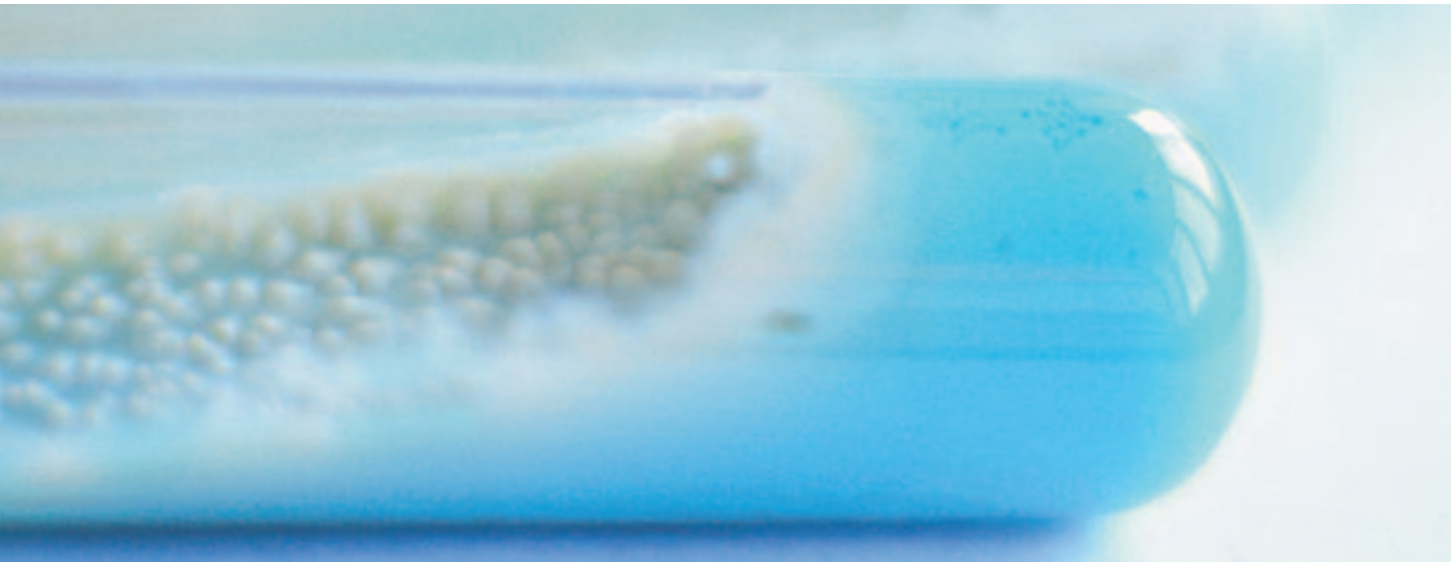
In 2008 FAPESP will invest US$ 388 million in fellowships and research grants. The success rate for proposals in the fellowship programs ranges from 40 per cent to 63 per cent. In the grants programs the proposal success rate ranges from 40 per cent to 60 per cent, depending on the particular type of grant.

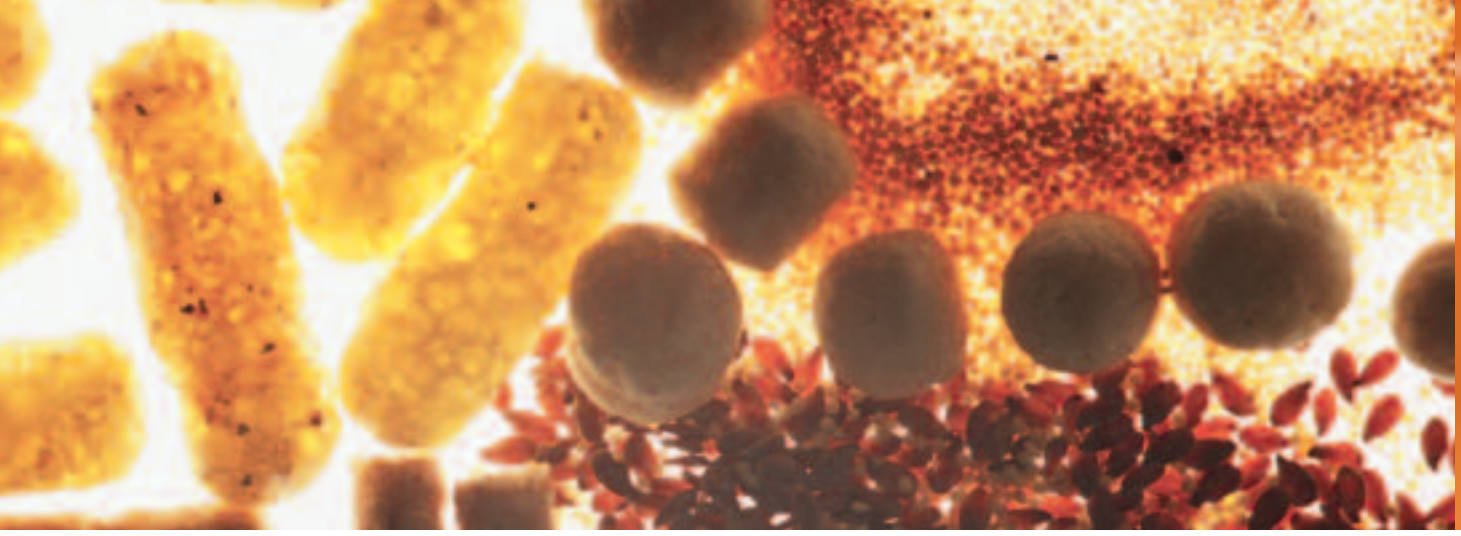
OPPORTUNITIES AND CHALLENGES

One of FAPESP's goals is the broadening and diversification of the research system in the state of São Paulo, strengthening the existing centers of excellence, by supporting their research, and stimulating the creation of new centers or research groups tackling new lines of activity. This is achieved mainly by funding Young Researchers Awards, the Biota-FAPESP Program, RIDC (Research, Innovation and Dissemination Centers) Program and the Thematic Projects.

All of these have in their teams, in addition to experienced scientists, young researchers as post-doctoral fellows, from Brazil and from abroad. FAPESP supports more than one thousand post-doctoral fellowships.

Contact FAPESP (www.oportunidades.fapesp.br) or a coordinator from the Thematic Project which interests you and see how to obtain a post-doctoral internship.





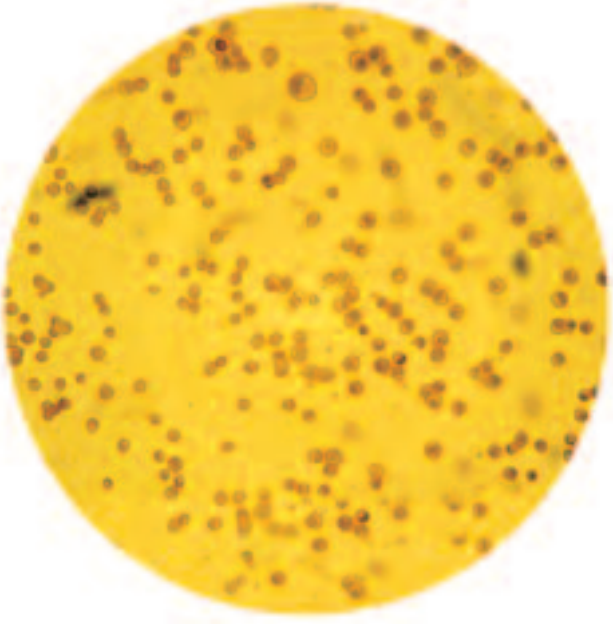
RESULTS OF GREAT IMPACT

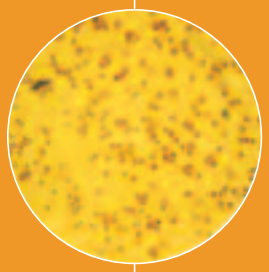
When the research program for Thematic Projects was created, in 1990, FAPESP's objective was to provide a qualitative leap in Brazilian scientific research and meet the state of São Paulo's own particular demands for development. Since then, 1,100 projects in all fields of knowledge have been selected and supported. Selection is through a stringent peer reviewing process, using multiple reviewrs for each proposal.

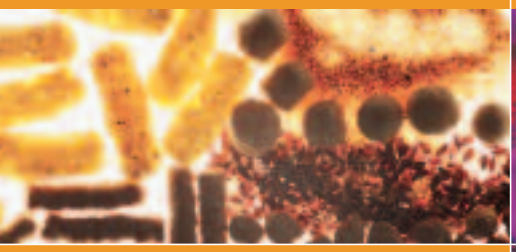
Thematic Projects are characterized by the breadth of their research and the boldness of their objectives. They are supported for four years (as opposed to two years for a regular research grant) and are lead by teams of experienced researchers.

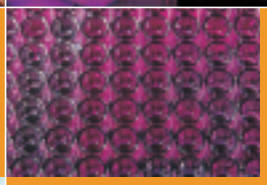
Thematic Projects are funded, on the average, with 450 thousand dollars, plus fellowships. The salaries for the investigators and staff are not included in this amount since in Brazil they are paid by their universities. Each project is lead by 3 PI's and involves several undergraduate and graduate students.

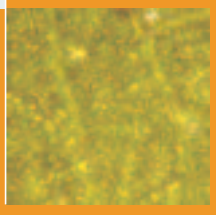
Thematic Projects create opportunities for scientists in São Paulo to advance knowledge by creating internationally competitive science, while, simultaneously, educating a new generation of researchers.











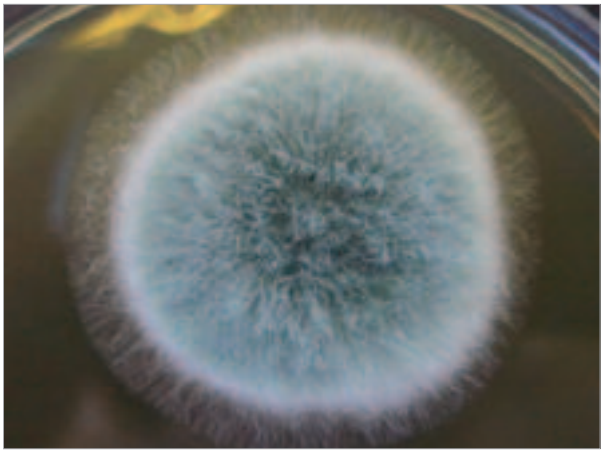
FAPESP
THE STATE OF SÃO PAULO
RESEARCH FOUNDATION

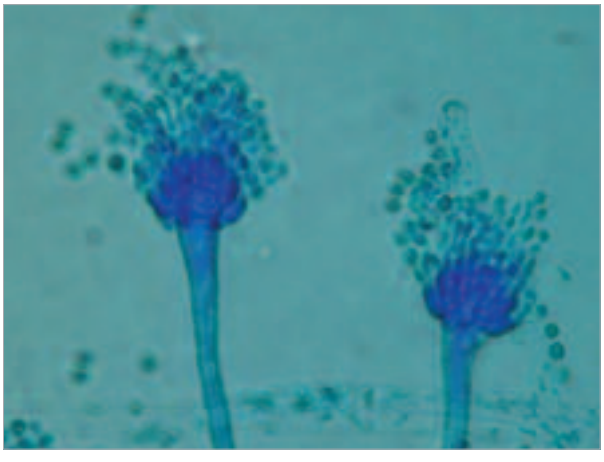
RUA PIO XI, 1500, ALTO DA LAPA
CEP: 05468-901 - SÃO PAULO - SP - BRASIL
FONE: 55.11.3838-4000

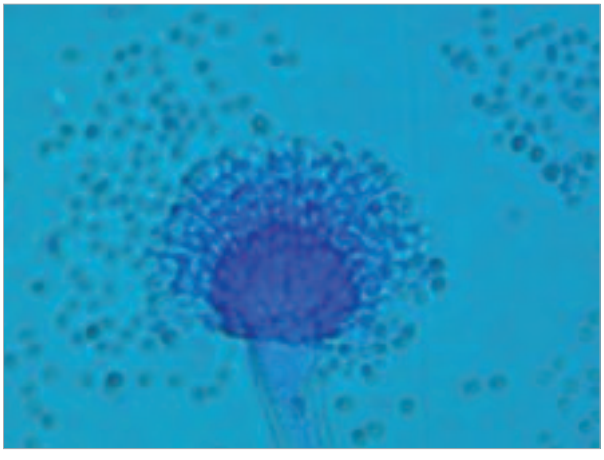
MOLECULAR ANALYSIS OF CALCINEURIN IN HUMAN PATHOGENIC FUNGI

Gustavo Henrique GOLDMAN

Ribeirão Preto School of Pharmaceutical Sciences / University of São Paulo (USP)







(A) A four day A. fumigatus *culture on malt extract agar (above). Light microscopy pictures are taken at 1000x, stained with lacto-phenol cotton blue (right). (B) and (C) show details of the asexual structure named conidiophore*

Environmental sensing and the retrieval of essential nutrients from the environment are general metabolic traits that are associated with the growth of *P. brasiliensis* and *A. fumigatus* in an inhospitable environment such as its human host. Calcium is a ubiquitous second messenger that functions in signal transduction pathways in eukaryotic organisms. Calcium signaling is important for several cellular processes, including fertilization and development, exocytose, muscle contraction, motility, chemotaxis, cell division, differentiation, programmed cell death and chromatin remodeling in multicellular eukaryotes. Our project proposes to study the system of calcineurin regulation in two human pathogenic fungi, *P .brasiliensis* and *A. fumigatus*, and the relationship between this system and two essential aspects of pathogenicity in these organisms: 1) the mycelium-to-yeast transition in *P. brasiliensis* and 2) the sensing of the human host environment and the nutrient acquisition in *A. fumigatus*. In the last four years, our group has been actively working with both pathogenic fungi, developing a series of resources and molecular tools aiming to understand the mechanisms of virulence/pathogenicity in these organisms. Thus, the main objectives of this proposal are: 1) to analyze microarray gene expression of the *P. brasiliensis* mycelium-to-yeast transition blocked by cyclosporine; 2) by using yeast two-hybrid screening, to identify cDNAs that correspond to genes that encode *P. brasiliensis* proteins that interact with calcineurin during the mycelium-to-yeast transition; 3) construction of a vector for transformation and RNAi inhibition in *P. brasiliensis*; 3) molecular characterization of an *A. fumigatus* gene that encodes the homologue of the *Crz*1 (*CrzA*) transcription factor that is transported to the nucleus after dephosphorylation by calcineurin; 5) to identify genes that suppress by high copy number the absence of *CalA* e *CrzA* and microarray gene expression analysis of the wild type and *calA* e *crzA* mutants in different stressing conditions; 6) characterization of genes involved in the inorganic phosphate utilization and their relationship with *calA* and *crzA* mutant; and 7) molecular characterization of *A. fumigatus* genes that encode proteins with Ca^{2+}-binding domains (EF-hands).

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Calcium is a ubiquitous second messenger that functions in signal transduction pathways in eukaryotic organisms. Our project proposes to study the system of calcineurin regulation in two human pathogenic fungi, *Aspergillus fumigatus* and *Paracoccidioides brasiliensis*. The protein phosphatase calcineurin is an important mediator connecting calcium-dependent signaling to various cellular responses in multiple organisms. In fungi calcineurin acts largely through regulating Crz1p-like transcription factors. We characterized an *Aspergillus fumigatus Crz1* homologue, *CrzA*, and demonstrated its mediation of cellular tolerance to increased concentrations of calcium and manganese. In addition to acute sensitivitiy to these ions, and decreased conidiation, the *crzA* null mutant suffered altered expression of calcium transporter mRNAs under high concentrations of calcium, and loss of virulence when compared to the corresponding complemented and wild type strains. We used multiple expression analyses to probe the transcriptional basis of *A. fumigatus* calcium tolerance by identifying several genes having *calA* and/or *crzA* dependent mRNA accumulation patterns. We also demonstrated that contrary to previous findings, the gene encoding the *A. nidulans* calcineurin subunit homologue, *CnaA*, is not essential, and that the *cnaA* deletion mutant shared the morphological phenotypes observed in the corresponding *A. fumigatus* mutant, *△calA*. By then exploiting the *A. nidulans* model system, we have linked calcineurin activity with asexual developmental induction, finding that CRZA supports appropriate developmental induction in a calcineurin and *BrlA*-dependent manner in both species.

Not only is our project related to calcium metabolism in *A. fumigatus*, but we are also using genomic tools in collaboration with several groups around the world trying to understand the biology of this organism. Recently, we presented the genome sequences of a new clinical isolate of *A. fumigatus*, A1163, and two closely related but rarely pathogenic species, *Neosartorya fischeri* NRRL181 and *A. clavatus* NRRL1. Cross-species comparison has revealed that 8.5%, 13.5% and 12.6%, respectively, of *A. fumigatus*, *N. fischeri* and *A. clavatus* genes are species-specific. These genes are significantly smaller in size than core genes, contain fewer exons and exhibit a subtelomeric bias. We extended these studies by identifying fungal attributes preferentially employed during adaptation to the mammalian host niche generating multiple genome-wide gene expression profiles from minute samplings of *A. fumigatus* germlings during initiation of murine infection. As functions of phylogenetic conservation and gene localisation, around 28 and 30 per cent, respectively, of the entire *A. fumigatus* subtelomeric and lineage-specific gene repertoire is induced relative to laboratory culture, and physically clustered genes including loci directing pseurotin, gliotoxin and siderophore biosyntheses are a prominent feature.

MAIN PUBLICATIONS

Soriani FM, Malavazi I, Ferreira MES, Savoldi M, Kress MR, Goldman MHS, Loss O, Bignell E, Goldman GH. 2008. Functional characterization of the *Aspergillus fumigatus CRZ1* homologue, *CrzA*. *Molecular Microbiology*. **67**:1274-1291.

Fedorova ND, Khaldi N, Joardar V, Maiti R, Amedeo P, Anderson MJ, Crabtree J, Silva JC, Badger JH, Albarraq A, Angiuoli S, Bussey H, Bowyer P, Cotty PJ, Dyer PS, Egan A, Galens K, Fraser C, Haas B, Inman J, Kent R, Lemieux S, Malavazi I, Orvis1 J, Roemer T, Ronning CM, Sundaram1 JP, Sutton G, Turner G, Venter JC, White OR, Whitty BR, Youngman P, Wolfe KW, Goldman GH, Wortman JR, Jiang B, Denning DW, Nierman WC. Genomic Islands in the Pathogenic Filamentous Fungus *Aspergillus fumigatus*. *PLOS Genetics*. (in press).

Gustavo Henrique GOLDMAN

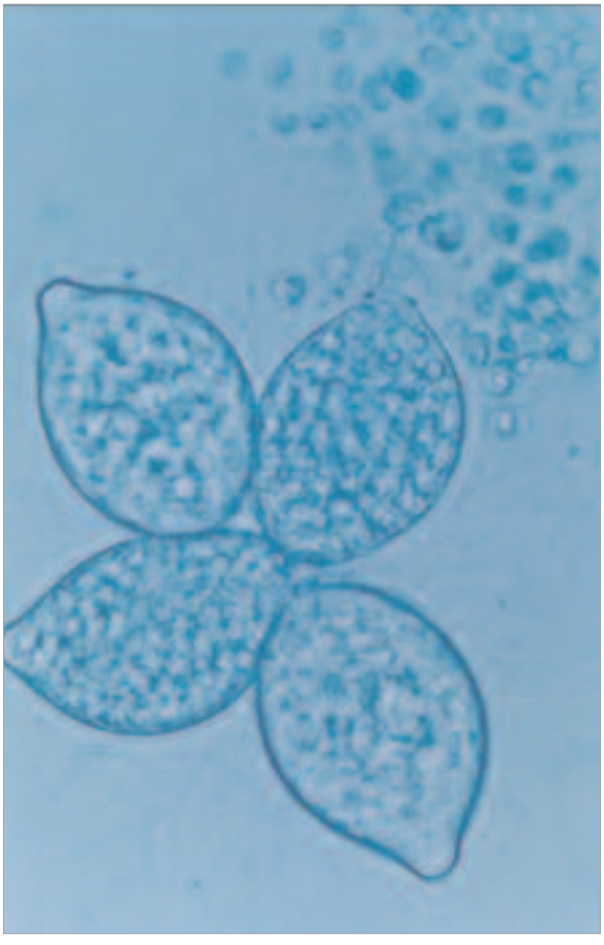
Faculdade de Ciências Farmacêuticas de Ribeirão Preto / Universidade de São Paulo (USP)
Departamento de Ciências Farmacêuticas
Avenida do Café, S/N – Monte Alegre
CEP 14040-903 – Ribeirão Preto, SP – Brasil

+55-16-3602-4280
ggoldman@usp.br

GENIC EXPRESSION REGULATION IN MICROORGANISMS

Suely Lopes GOMES

Chemistry Institute / University of São Paulo



Sporulation of B. emersonii

This research project aims at investigating the molecular aspects related to the control of gene expression during cell differentiation, heat shock and other stress conditions utilizing as model systems: the gram-negative bacterium *Caulobacter crescentus* and the chytridiomycete *Blastocladiella emersonii*. In *Caulobacter crescentus*, we intend to investigate a putative homologue of the gene *hspR* (CC0081), which in other bacteria encodes a repressor that controls the expression of several heat shock genes. The HSPR protein represses gene expression by binding to an inverted repeat present in the regulatory region of the genes, denominated HAIR (HspR associated inverted repeat). For these studies we will construct a null mutant in gen Cc0081, whose phenotype will be analyzed with respect to different kinds of environmental stresses. Another objective of this project deals with the characterization of 5 ECF (extracytoplasmic function) sigma factors from *Caulobacter*: *SigF, SigU, SigT, SigE* and *SigR*. Concerning *SigF*, which we recently showed to be involved in the response to hydrogen peroxide in stationary phase cells, we will investigate the molecular mechanisms controlling its activity. Preliminary results indicate that *SigF* is post-transcriptionally regulated during stationary phase. In addition, gene Cc3252, which forms with *SigF* a probable operon, will be investigated as a possible anti-sigma factor. *SigU* and *SigT*, whose expression is induced by osmotic stress, will be better characterized, looking for *Caulobacter* genes that are regulated by these sigma factors. Null mutants of *SigE* and *SigR* will be obtained and their phenotypes analyzed, to uncover the role of these sigma factors in the control of gene expression in this bacterium. We also propose to characterize expressed sequence tags (ESTs) from *Blastocladiella emersonii* exposed to heat shock or Cadmium. In the case of exposure to Cadmium, very little is known with respect to the primary targets of this heavy metal. With the use of *Blastocladiella* cDNA microarrays, recently constructed in our laboratory, we intend to analyse the global gene expression of this fungus during its two differentiation phases: the sporulation and the germination. We also intend to investigate *B. emersonii* genes expressed under different nutritional growth conditions. One of the objectives of the project is to characterize cyclin dependent kinases (Cdks) in *B. emersonii*, and to investigate their expression during the sporulation and germination stages. Taking into account preliminary results, we intend to characterize two kinases with non conserved PST AIRE motif (cyclin dependent kinases non PST AIRE) found in *B. emersonii*. In addition, we intend to screen different *B. emersonii* cDNA libraries looking for sequences encoding other putative Cdks for their characterization. Mapping and sequencing of the mitochondrial genome of *Blastocladiella*, aiming a phylogenetic study, will be also carried out during this project.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In *Caulobacter crescentus*, we are investigating the role of ECF sigma factors, which are involved in bacterial responses to distinct types of environmental stresses. The *Caulobacter* genome possesses 13 genes encoding sigma factors of this family. We have up to now characterized 4 of these factors by constructing null mutants of their genes, transcription fusions with their promoters, and through micro array experiments. Our data showed that the ECF sigma factor *SigF* is involved in the response to oxidative stress in stationary phase cells, while *SigT* and *SigU* have a role in osmotic and oxidative stress in the exponential growth phase. Our results also showed that *SigR* is important for the bacterial response to singlet oxygen. Furthermore, we observed that *SigF* is posttranscriptionally controlled, whereas *SigT* and *SigU* are transcriptionally regulated during stress. On the other hand, *SigR* is controlled both transcriptionally and posttranscriptionally.

Caulobacter crescentus *cell cycle*

In *Blastocladiella emersonii*, we are studying differentially expressed genes during the life cycle of the fungus and genes responding to different types of environmental stresses. The construction of cDNA libraries with RNAs isolated from cells at different stages of development or subject to stresses like heat shock and cadmium, followed by large scale sequencing of ESTs, revealed over 6,000 putative distinct genes from this fungus. About 3,700 of these genes were amplified to construct cDNA microarrays which are being used in global transcriptome studies of cells growing under different conditions. Cyclin dependent kinases of the fungus are being also characterized, with the identification of three distinct cDNAs encoding this type of kinases. Analysis of the expression of their genes, associated with determination of their kinase activity during the life cycle is being carried out to uncover their roles in the fungus.

MAIN PUBLICATIONS

Alvarez-Martinez CE, Baldini RL, Gomes SL. 2006. A *Caulobacter crescentus* extracytoplasmic sigma factor mediating the response to oxidative stress in stationary phase. *Journal of Bacteriology.* **188(5)**: 1835-1846.

Susin MF, Baldini RL, Gueiros-Filho F, Gomes SL. 2006. GroES/GroEL and DnaK/DnaJ have distinct roles in stress responses and during cell cycle progression in *Caulobacter crescentus*. *Journal of Bacteriology.* **188(23)**: 8044-8053.

Ribichich KF, Georg RC, Gomes SL. 2006. Comparative EST analysis provides insights into the basal aquatic fungus *Blastocladiella emersonii*. *BMC Genomics* **12**: 177.

Koide T, Salem-Izacc SM, Gomes SL, Vêncio RZN. 2006. SpotWhatR: a user-friendly microarray data analysis system. *Genetics and Molecular Research.* **5(1)**: 93-107.

Vêncio RZN, Koide T, Gomes SL, Pereira CA. 2006. BayGO: Bayesian analysis of ontology term enrichment in microarray data. *BMC Bioinformatics.* **7**: 86.

Georg RC, Gomes SL. 2007. Transcriptome analysis in response to heat shock and cadmium in the aquatic fungus *Blastocladiella emersonii*. *Eukaryotic Cell* **6(6)**:1053-1062, 2007.

Alvarez-Martinez CE, Lourenço RF, Baldini RL, Laub MT, Gomes SL. 2007. The ECF sigma factor ÛT is involved in osmotic and oxidative stress response in *Caulobacter crescentus*. *Molecular Microbiology* **66(5)**: 1240-1255.

Georg RC, Gomes SL. 2007. Comparative expression analysis of members of the Hsp70 family in the chytridiomycete *Blastocladiella emersonii*. *Gene.* **386(1-2)**: 24-34.

Pugliese L, Georg RC, Fietto LG, Gomes SL. 2008. Expression of genes encoding cytosolic and endoplasmic reticulum HSP90 proteins in the aquatic fungus *Blastocladiellla emersonii*. *Gene.* **411(1-2)**: 59-68.

Suely Lopes GOMES

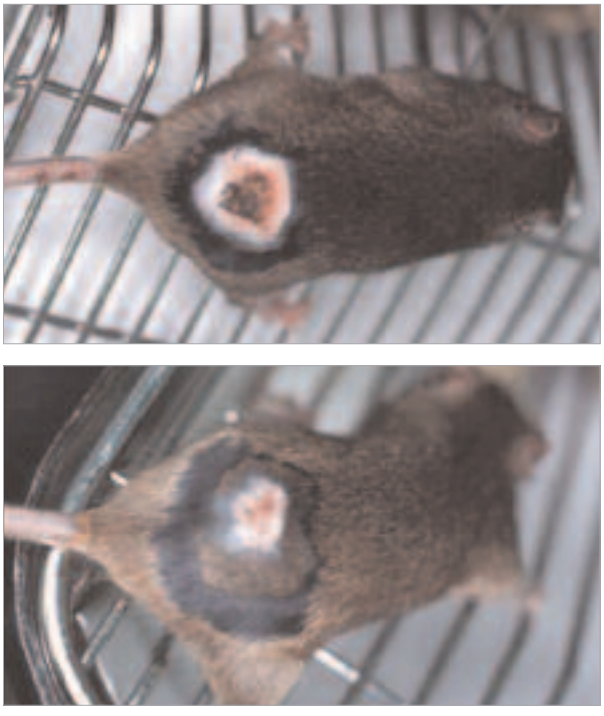
Instituto de Química
Universidade de São Paulo (USP)
Departamento de Bioquímica
Avenida Prof. Lineu Prestes,748 – Butantã
05508-900 – São Paulo, SP – Brasil

+55-11-3091-3826
sulgomes@iq.usp.br

DNA REPAIR GENES: FUNCTIONAL ANALYSIS AND EVOLUTION

Carlos Frederico Martins MENCK

Institute of Biomedical Sciences / University of São Paulo (USP)



The mouse above, knockout mice (XPAKO) submitted to skin gene therapy with recombinant adenoviruses. Note that the animal that has been infected with the AdXPA virus (below) recovered faster from UV-B induced irradiation

Most of our research activities are based on the understanding of DNA repair and mutagenesis mechanisms in mammalian cells, as well as other organisms. With the recent enormous amount of data generated by genomic approaches, our project is including now an evolutionary point of view. In this Thematic Proposal we present 4 different subprojects, and 2 of them are based on studies of DNA repair in mammalian cells, giving priorities to primary cells from human beings. Much of this work will employ the well-succeeded recombinant adenovirus vectors, which were developed in this laboratory. These, carrying DNA repair and DNA repair-related genes, will be used to complement cells with human syndromes that present DNA repair deficiencies (such as xeroderma pigmentosum). The vectors can revert completely cells deficiencies and we are now initiating studies with the perspectives to analyze directly their ability to complement knockout mire for XP genes. Moreover, these vectors will also be used in order to diagnose and identify the genes involved in XP patients as well as in the studies of the dynamics of DNA repair proteins in the cells. Another endpoint to be investigated in mammalian cells is apoptosis induced by DNA damage, as we are trying to decipher the signals that lead the reli to trigger its program of cell death. Adenovirus vectors bearing the photolyase genes are being constructed and they will be tested on their ability to prevent apoptosis in human cells deficient for DNA repair. The involvement of RNA transcription, DNA replication, PARP and cell cycle in signaling for apoptosis in DNA damaged cells are also being investigated. In a different subproject, we are trying to understand the how plant cells defend their genomes from the presence of DNA damage. Two genes that were previously cloned (*AtXPB*1 e *Thi*1) have being targeted in this work, and our plan is to identify the roles of the proteins encoded by these genes, in the repair of DNA damage in nuclei (*AtXPB*) and organels (*Thi*1). We also plan to understand how these genes are expressed in plants. Finally, based on the knowledge of the complete genome sequence of *Caulobacter crescentus*, we intend to identify and investigate the genes involved in the DNA repair of this alpha proteobacteria. Basically, we propose to construct and analyze the phenotype of bacterial mutants on DNA repair related genes. The possibility to synchronize these bacteria may be useful to ascertain the effect of cell cycle on the repair functions in these cells. The differential gene expression along the cells cycle can also be investigated. To our knowledge, this is the first proposal for a genomic approach to investigate DNA repair in bacteria different from the classic *Escherichia coli* model. Subproject 1: Recombinant genetic vectors to study DNA repair in mammalian cells. Subproject 2: Signaling to apoptosis in mammalian cells with damaged genome. Subproject 3: DNA repair genes in plants and functional analysis of the genes *AtXPB*1 and *Thi*1. Subproject 4: Identification and function of genes related to DNA repair in *Caulobacter crescentus*.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

This Project basically aims at understanding how cells respond to and protect their genomes from environmental aggressions. The impact of these protection systems is dramatically illustrated with the human genetic syndrome xeroderma pigmentosum (XP). XP patients present high frequency of skin cancers in regions exposed to sunlight, early aging and developmental problems, which are mainly due to DNA repair defects in their cells. In this Project, we succeeded in developing recombinant adenovirus vectors which correct the genetic defects of the XP cells. This has been achieved both *in vitro* and *in vivo* by using XP-knockout mice, which simulate the XP syndrome. These adenoviruses were shown to prevent the appearance of skin tumors in mice irradiated with UVB light, opening potential gene therapy perspectives for these patients. More recently, work with vectors carrying genes of photolyases (specific DNA repair enzymes) has enabled us to identify the types of lesions that promote apoptosis by UV, thus answering a longstanding question. Interestingly, XP cells respond to a different lesion when compared to normal human cells, which indicates that the XP problem may be not only related to a quantitative number of unrepaired lesions, but also to different type of lesions.

In a different approach, we have been investigating the nature of the signaling process that lead to cell death after the induction of DNA lesions. The results clearly indicate the role of DNA replication as an important sensor mechanism that makes the cells respond to genetic damages. Also, in collaboration with Dr. Bernd Kaina (Mainz, Germany), we have identified DNA repair pathways involved in the protection of cells' genomes to DNA lesions induced by chemotherapeutic agents, with potential implications in tumor treatment.

Besides, DNA repair genes are highly conserved throughout evolution, and this led us to make significant and pioneer contributions in the identification of DNA repair genes in plants, homologs to those deficient in XP patients. In bacteria, we also were pioneer in the use of the bacterial model *Caulobacter crescentus* in studies of DNA repair mechanisms. These studies resulted in the identification of functions for a previously uncharacterized bacterial operon, which codes for a complex of proteins responsible for a translesion DNA polymerase role in the cell. Finally, studies with the genomes of the bacteria from the group of xanthomonadales led to the identification of several genomic islands, potentially originated from horizontal gene transfer. The beauty of the work is that the methodology employed allowed to determine that some of these islands were transferred a long time ago, and carry genes involved in the primary metabolism, adding essential genes to the list of genes that may be exchanged between bacteria during evolution.

MAIN PUBLICATIONS

Marchetto MCN*, Muotri AR*, Burns DK, Friedberg EC, Menck CFM. 2004. Gene transduction in skin cells: preventing cancer in xeroderma pigmentosum mice. *Proc. Natl. Acad. Sci*. USA, **101(51)**: 17759-17764.
*both authors have equally contributed to this work.

Morgante PG*, Berra CM*, Nakabashi M, Costa RMA, Menck CFM, Van Sluys MA. 2005. Functional *XPB/RAD25* redundancy in *Arabidopsis* genome: characterization of *AtXPB2* and expression analysis. *Gene*. **344**: 93-103.
*both authors have equally contributed to this work.

Lima-Bessa KM, Menck CFM. 2005. Skin cancer: lights on genome lesions. *Current Biology*, **15(2)**: R58-61.

Ribeiro DT, Farias LP, Almeida JD, et al. 2005. Functional characterization of the *Thi*1 promoter region from *Arabidopsis thaliana*. *Journal Experimental Botany*. **56(417)**:1797-1804.

Galhardo RS, Rocha R, Marques MV, Menck CF. 2005. A SOS-regulated operon involved in damage-inducible mutagenesis in *Caulobacter crescentus*. *Nucleic Acids Research*. **33(8)**: 2603-2614.

Batista LFZ, Chiganças V, Brumatti G, Amarante-Mendes GP, Menck CFM. 2006. Involvement of DNA replication in ultraviolet-induced apoptosis of mammalian cells. *Apoptosis* **11(7)**:1139-1148.

Godoi PH, Galhardo RS, Luche DD, Van Sluys MA, Menck CF, Oliva G. 2006. Structure of the Thiazole Biosynthetic Enzyme THI1 from *Arabidopsis thaliana*. *J. Biol. Chem.* **281(41)**: 30957-30966.

Martins-Pinheiro M, Marques RCP, Menck CFM. 2007. Genome analysis of DNA repair genes in the alpha proteobacterium *Caulobacter crescentus*. *BMC Microbiology*. **7**: 17.

Lima-Bessa KM, Armelini MG, Chiganças V, et al. 2008. CPDs and 6-4PPs play different roles in UV-induced cell death in normal and NER-deficient human cells. *DNA Repair* **7(2)**: 303-312.

Lima WC, Paquola AC, Varani AM, Van Sluys MA, Menck CF. 2008. Laterally transferred genomic islands in Xanthomonadales related to pathogenicity and primary metabolism. *FEMS Microbiol Lett*. **281(1)**: 87-97.

Carlos Frederico Martins MENCK

Instituto de Ciências Biomédicas
Universidade de São Paulo (USP)
Departamento de Microbiologia
Avenida Prof. Lineu Prestes, 1374 – C. Universitária
05508-900 – São Paulo, SP – Brasil

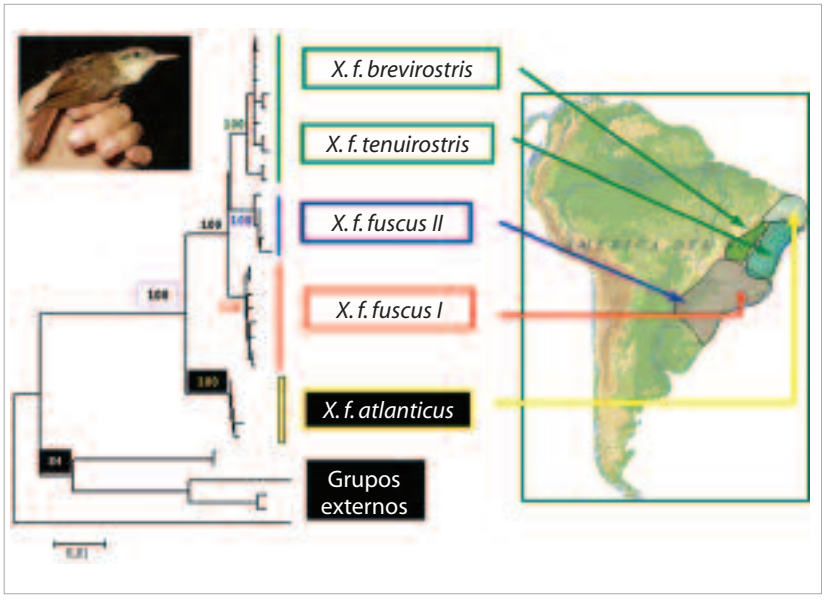
+55-11-3091-7499
cfmmenck@usp.br

RECONSTRUCTION OF THE EVOLUTIONARY HISTORY AND PHYLOGENETIC STUDIES OF NEOTROPICAL BIRDS BASED ON MOLECULAR MARKERS

Cristina Yumi MIYAKI

Institute of Biosciences / University of São Paulo (USP)

The Neotropics is a mega diverse region. The origin of such richness may be related to the geologic and climatic changes that occurred through time and its conservation depends on combined efforts. Birds comprise one of the most diverse groups of neotropical animals. Their endemism patterns and the association between the systematic and biogeographic relationships are appropriate for historical biogeography studies. Their occurrence in all biomes is also suitable to study the origin of diversity in the Neotropics. In the present research project, molecular techniques have been applied in phylogenetic and phylogeographic studies of birds. We will enhance taxa sampling of three groups which we have been studying (parrots, cracids, and toucans) and will include another one (hawks and eagles). We will also start the study of passerines from the Atlantic Forest to understand the biogeography and phylogeography of such taxa. The dates of divergence between lineages will be estimated, taxonomy uncertainties may be resolved, and the level of isolation between populations will be determined. These data will contribute to a better knowledge of the origin, distribution, and maintenance of the biodiversity of the Neotropics.



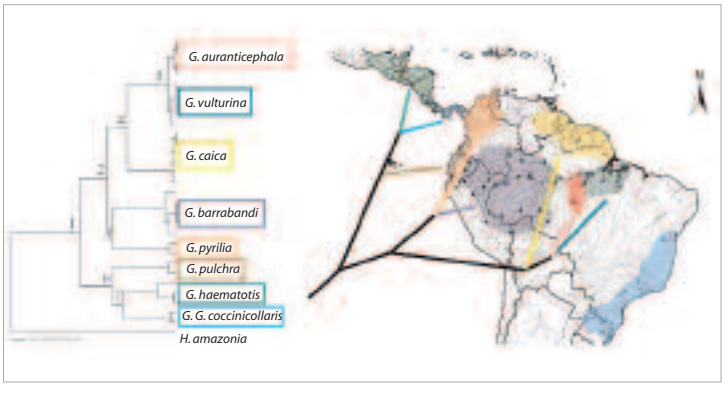
Relationships and geographic distribuitions of generic lineages of Xiphorhynchus fuscus

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The present project has been gathering data on the historic biogeography of birds and the biomes where they occur based on their phylogenies and population structure studies. The reconstructions of the evolutionary history of birds (including estimates of divergence dates of lineages) are used to infer possible factors that can be related to these divergences. These analyses suggest that past climate and geological changes in South America contributed to speciation events and intra-specific diversification. The results indicate a complex biogeographical history in the Neotropical region in contrast with less complex patterns in the Neartic and Paleartic regions. Also, our data indicate that taxonomic revisions are needed in some groups. Finally, we found taxa previously undescribed by Science and among them, there are populations that need conservation actions. We have counted on many collaborators to develop these subprojects.

Another main area of action of the present project is to provide genetic data for the management of endangered avian species. Given the complexity of conservation, we have been working with various collaborators. We have been recommending actions for captive programs and for diminishing the illegal traffic of wildlife.

Molecular phylogeny of the genus Gypopsitta and corresponding areas of distribuition of its species



MAIN PUBLICATIONS

Tavares ES, Yamashita C, Miyaki CY. 2004. Phylogenetic relationships among some neotropical parrot genera (Psittacidae, Aves) based on mitochondrial sequences. *Auk - American Journal of Ornithology*. **121(1)**: 230-249.

Grau ET, Pereira SL, Silveira LF, et al. 2005. Molecular phylogenetics and biogeography of Neotropical piping guans (Aves: Galliformes): *Pipile Bonaparte*, 1856 is synonym of *Aburria Reichenbach*, 1853. *Molecular Phylogenetics and Evolution.* **35**: 637-645.

Kimura RK, Pereira SL, Grau ET, Höfling E, Wajntal A. 2004. Genetic distances and phylogenetic analysis suggest that *Baillonius Cassin*, 1867 is a Pteroglossus Illiger, 1811 (Piciformes: Rhamphastidae). *Ornitologia Neotropical.* **15**: 527-537.

Ribas CC, Gaban-Lima R, Miyaki CY, Cracraft J. 2005. Historical biogeography and diversification within the Neotropical parrot genus "Pionopsitta" (Aves; Psittacidae). *Journal of Biogeography.* **32**:1409-1427.

Amaral FSR, Miller MJ, Silveira LF, Bermingham E, Wantal A. 2006. Polyphyly of the hawk genera Leucopternis and Buteogallus (Aves, Accipitridae): multiple habitat shifts during the Neotropical buteonine diversification. *BMC Evolutionary Biology.* **6**:10.

Tavares ES, Baker AJ, Pereira SL, Miyaki CY. 2006. Phylogenetic relationships and historical biogeography of Neotropical parrots (Psittaciformes: Psittacidae: Arini) inferred from mitochondrial and nuclear DNA sequences. *Systematic Biology.* **55**: 454-470.

Cabanne GS, Santos FR, Miyaki CY. 2007. Phylogeography of Xiphorhynchus fuscus (Passeriformes: Dendrocolaptidae): vicariance and recent demographic expansion in the southern Atlantic forest. *Biological Journal of the Linnean Society.* **91**: 73-84.

Ribas CC, Moyle RG, Miyaki CY, Cracraft J. 2007. The origin and diversification of montane lineages of Pionus parrots are causally linked to Andean tectonic uplift and climate change. *Proceedings of the Royal Society of London.* **1624**: 2399-2408.

Caparroz R, Leite KCE, Chinalia LA, Miyaki CY, et al. 2007. Characterization of microsatellite loci in three species of Amazona (Psittaciformes) using heterologous primers. *Ornitología Neotropical.* **18**: 439-444.

Cristina Yumi MIYAKI

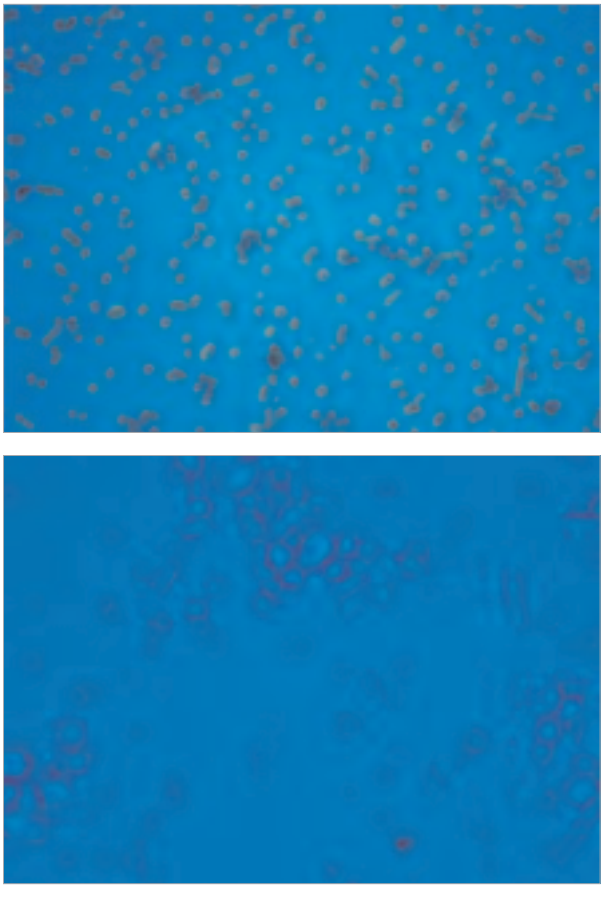
Instituto de Biociências
Universidade de São Paulo (USP)
Rua do Matão, 277 – CP 11461 – C. Universitária
CEP 05508-090 – São Paulo, SP – Brasil

+55-11-3091-7582
cymiyaki@ib.usp.br

MOLECULAR GENETICS AND FUNCTIONAL GENOMICS OF FUNGI

Nilce Maria Martinez ROSSI

Ribeirão Preto Medicine School / University of São Paulo (USP)



Microphotographs of microconidia (a) and arthroconidia (b) of Trichophyton sp.

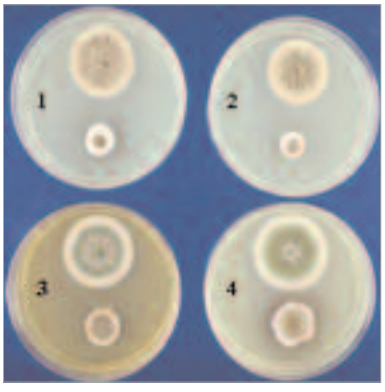
Dermatophytosis are superficial mycoses caused by a group of filamentous fungi called dermatophytes, which are able to invade keratinized substrates such as hair, nails and stratum corneum. Trichophyton rubrum, an anthropophilic and cosmopolitan fungus, is the most common agent of superficial mycoses, causing rarely deep dermatophytosis in imunocompromised hosts.

The regulation of gene expression is vital for all organisms and is especially required by fungi for rapid adaptation to cellular stress conditions, as is the case when they infect a host or when they are submitted to antifungal drugs. These adaptive mechanisms are extremely complex and most of them have not been fully clarified. The objective of the present project is to elucidate the metabolic pathways or cellular processes that permit fungi to survive under adverse conditions. In addition, we intend to identify genes that may be involved in pathogenicity and that therefore may become targets for the development of new antifungal drugs.

A determinant factor in the invasion and utilization of host tissue by fungi is the secretion of enzymes, which is frequently regulated by ambient pH. Consequently, the mechanisms of pathogenicity or even of resistance to inhibitors are likely to depend directly or indirectly on ambient pH monitoring. Thus, knowledge of the mechanisms that regulate the expression/secretion of enzymes involved in pathogenicity and of enzymes responsible for resistance to inhibitors will be of fundamental importance in the search for new therapeutic strategies, in the revelation of new drug targets, and therefore in the control of microorganisms that are harmful to the man.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The fungi regulate many cellular events as a function of pH environment. This genetic mechanism has extensive practical application, including installation, development and maintenance of dermatophytes in the host. Genes preferentially expressed in pH acid were identified in *T. rubrum*, a condition which mimics that found in the human skin. We show that the secretion of queratinases (virulence factor) by *T. rubrum* is dependent on the gene *pacC*, because the knockout of this gene promoted the decreased secretion of these enzymes, affecting growth in fragments of human fingernail. In addition, we revealed three molecular mechanisms of terbinafine resistance in fungi, an antifungal widely used for the treatment of dermatophytosis: a) the presence of multiple copies of a gene (*salA*) accelerates the metabolism of terbinafine; b) mutations in the squalene epoxidase gene cause resistance to this drug because it prevents the binding of terbinafine to squalene epoxidase, its target cell, and c) a transporter protein of *T. rubrum* participates in the terbinafine efflux. Our laboratory has also focused on the response to the stress caused by other drugs used to treat human mycoses, which have revealed new genes in dermatophytes, some of them potential targets for the development of new antifungal agents.



Colonial growth of the model fungus Aspergillus nidulans *showing phenotype changes dependent on the composition and pH of the culture medium. (http://www.fgsc.net/home_page_image_information.htm)*

Several methods have been standardized to study *T. rubrum* such as the electrophoretic molecular karyotype, the molecular transformation and gene knockout that will allow the functional analysis of virtually any gene of this dermatophyte. As a result of these findings, the group was invited to join the Steering Committee of the dermatophytes genome project developed by the consortium formed by The Broad Institute of Harvard and MIT and the NIH (National Institute of Health). The group was also invited by the editor of the journal Mycopathologia (Springer) to write a review about Antifungal resistance mechanisms in dermatophytes (DOI 10.1007/s11046-008-9110-7).

MAIN PUBLICATIONS

Fachin AL, Ferreira-Nozawa MS, et al. 2006. Role of the ABC transporter TruMDR2 in terbinafine, 4-nitroquinoline N-oxide and ethidium bromide susceptibility in *Trichophyton rubrum*. *J. Med. Microbiol.* **55**:1093-1099.

Ferreira-Nozawa MS, Silveira HC, Ono CJ, et al. 2006. The pH signaling transcription factor PacC mediates the growth of *Trichophyton rubrum* on human nail *in vitro*. *Med. Mycol.* **44**:641-645.

Graminha MA, Rocha EM, Prade RA, Martinez-Rossi NM. 2004. Terbinafine resistance mediated by salicylate 1-monooxygenase in *Aspergillus nidulans*. *Antimicrob. Agents Chemother*. **48**:3530-3535.

Gras DE, Silveira HC, Martinez-Rossi NM, Rossi A. 2007. Identification of genes displaying differential expression in the nuc-2 mutant strain of the mold *Neurospora crassa* grown under phosphate starvation. *FEMS Microbiol. Lett.* **269**:196-200.

Leal J, Squina FM, Martinez-Rossi NM, Rossi A. 2007 The transcription of the gene for iso-orotate decarboxylase (IDCase), an enzyme of the thymidine salvage pathway, is downregulated in the pregc mutant strain of *Neurospora crassa* grown under phosphate starvation. *Can. J. Microbiol.* **53**:1011-1015.

Maranhão FC, Paião FG, Martinez-Rossi NM. 2007. Isolation of transcripts over-expressed in human pathogen *Trichophyton rubrum* during growth in keratin. *Microb. Pathog*. 43:166-172.

Martinez-Rossi NM, Peres NTA, Rossi A. 2008. Antifungal resistance mechanisms in dermatophytes. *Mycopathologia*. DOI 10.1007/s11046-008-9110-7.

Paião FG, Segato F, Cursino-Santos JR, Peres NT, Martinez-Rossi NM. 2007. Analysis of *Trichophyton rubrum* gene expression in response to cytotoxic drugs. *FEMS Microbiol. Lett.* **271**:180-186.

Rocha EM, Gardiner RE, Park S, Martinez-Rossi NM, Perlin DS. 2006. A Phe389Leu substitution in ErgA confers terbinafine resistance in *Aspergillus fumigatus*. *Antimicrob. Agents Chemother.* **50**:2533-2536.

Segato F, Nozawa SR, Rossi A, Martinez-Rossi NM. 2008. Over-expression of genes coding for proline oxidase, riboflavin kinase, cytochrome c oxidase and an MFS transporter induced by acriflavin in *Trichophyton rubrum*. *Med. Mycol.* DOI: 10.1080/13693780701742381.

Nilce Maria Martinez ROSSI

Faculdade de Medicina de Ribeirão Preto
Universidade de São Paulo (USP)
Avenida Bandeirantes, 3900 – Monte Alegre
CEP 14049-900 – Ribeirão Preto, SP – Brasil

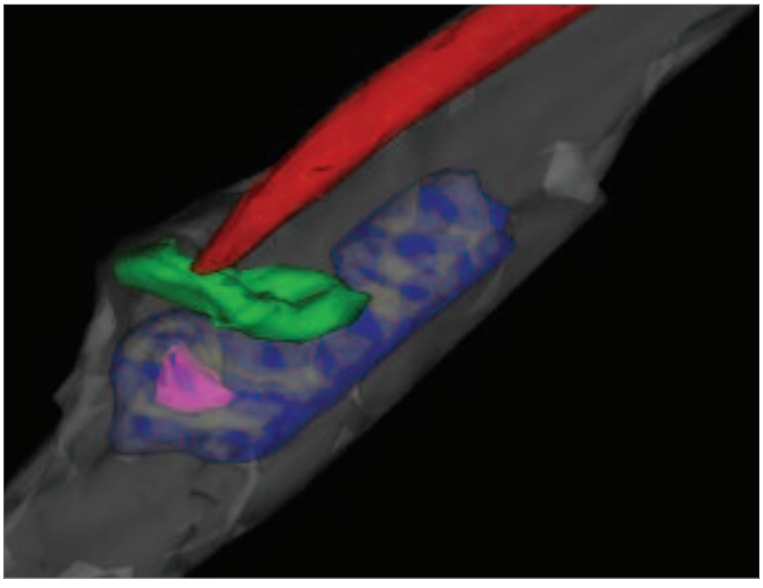
+55-16-3602-3150
nmmrossi@usp.br

NUCLEAR STRUCTURE AND CELLULAR ORGANIZATION IN THE DIFFERENTIATION AND CELLULAR CYCLE OF *Trypanosoma cruzi*

Sérgio SCHENKMAN

Microbiology, Immunology and Parasitology Departament / Federal University of São Paulo (Unifesp)

The establishment of microbial infections depends of parasite adaptation and survival in their hosts. To survive, the *Trypanosoma cruzi*, a protozoan parasite, causative agent of Chagas disease, alternates between forms that divide in the insect vector intestinal lumen (epimastigote forms), or in mammalian cell cytoplasm (amastigote forms), to non-proliferative forms but capable of entering mammalian cells after being exposed to extreme conditions in the blood stream or insect feces. Transformation from one form the other involves complex mechanism poorly understood, by which the parasite sense the environmental conditions, progressing through the cell cycle, or undergoing differentiation. Identification of the signals and mechanisms that led to one or other process is therefore relevant to understand and control infection. In a previous project, our laboratory started studies about the nuclear organization of *T. cruzi* with the goal to understand the process involved in the gene expression control during the different developmental stages of the parasite. We found large changes in the nuclear structure by comparing replicative and non-replicative forms of this protozoan. These structural changes correlated with a general transcription shutdown [1] and changes in the chromatin structure and organization [2]. To further understand the meaning of these changes we localized the replication and transcription sites in the nuclei of this parasite. We found that DNA precursors were incorporated close to the nuclear envelope, and later moved to the nuclear interior [3], in agreement with the findings that they are in continuous movement and become restrained to the nuclear periphery to replicate [4]. By using antibodies specific for the RNA polymerase II large subunit of *T. cruzi*, characterized in our laboratory [5], we observed that transcription is also located in restricted domains, close to the nucleolus and that these domains disappeared when transcription was inhibited (in preparation). To understand these differential localization of replication and transcription, we started to investigate the structure and modifications in the chromatin, recognized to have a central role in the organization and coupling of nuclear events [6]. We found differential phosphorylation and acetylation of histones during the cell cycle and after differentiation of the parasite into non-replicative forms. Therefore, our main goal in this project is to further characterize these structural and functional changes in the T. cruzi nuclei during the cell cycle and differentiation. These studies may help us to understand how the parasite controls his life cycle.



T. cruzi *epimastigotes undergoing differentiation. The figure is a 3D reconstruction of serial sections obtained by transmission electron microscopy of a parasite attached to a slide. Grey areas represent the cell body, red the flagellum, green the kinetoplast, blue the nucleus containing the nucleolus in pink and the electron dense chromatin in grey. Magnification: 50000X.*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The major goal of our research has been the identification of novel mechanisms that would be useful as targets to combat parasitic diseases caused by trypanosomes. Gene transcription was found concentrated in a few nuclear regions, located in the nuclear interior of replicating forms of *Trypanosoma cruzi*, the agent of Chagas´ disease. These transcriptional regions disappear abruptly when infective and non-dividing trypomastigotes are formed. Chromosomes were found moving in the nuclear interior but during replication the chromatin is immobilized near the nuclear envelope. This nuclear organization was found dependent on histone modifications. Histone H1 was found phosphorylated in a cyclin-dependent kinase in *T. cruzi*. However, the *T. cruzi* histone H1 does not localize with the bulk of chromatin. It is rather concentrated in the nucleolus. Upon phosphorylation, occurring with the cell cycle progression, H1 dissociates from the nucleolus, suggesting that H1 is implicated in ribosomal RNA synthesis and/or processing regulation. Histones H4 and H2B were found mainly acetylated, while histones H3 and H2B methylated in *T. cruzi*. The acetylation sites were identified in the N-terminal portion of histone H4. Synthetic peptides with the identified modifications were used to obtain specific antibodies, which labeled nuclear domains involved specifically in the transcription, replication, or DNA repair. Insights about the mechanisms involved in the control of cell organization that could sense signals required for differentiation were also obtained. As trypanosomes decrease protein synthesis during differentiation, protein kinases involved in the regulation of protein translational initiation were studied. In our work a protein kinase involved in the phosphorylation of the eukaryotic initiation factor 2 (eiF2·) was characterized. This new kinase was detected in the flagellar pocket, suggesting that the cellular organization must be linked to the protein synthesis.

In a new program project we continued to work with the histone modifications and we started by looking the localization of several other kinases involved in the control of cellular morphogenesis. Kinases with characteristics of "Target of Rapamycin" (TOR) kinases, probably involved in the control of cellular growth were identified in trypanosomes. One of the kinases was found in the unique mitochondria of trypanosomes, while another, was found localized in vesicles similar to acidocalcisomes of these organisms. We are currently investigating their role in the control of cell cycle and differentiation.

In conclusion, our program projects provided some key features to start understanding the molecular mechanism controlling adaptative responses in protozoan parasites.

MAIN PUBLICATIONS

Ramirez MI, Yamauchi L, Freitas-Junior LHG, Uemura H, Schenkman S. 2000. The use of the green fluorescent protein to monitor and obtain more efficiently transfected *Trypanosoma cruzi*. *Mol. Biochem. Parasitol.* **111**:235-240.

Elias MCQB, Marques-Porto R, Freymuller E, Schenkman S. 2001. Transcription rate modulation through *Trypanosoma cruzi* life cycle is reflected by nuclear organisation. *Mol. Biochem. Parasitol*. **112**: 95-99.

Porto RM, Amino R, Elias MC, Faria M, Schenkman S. 2002. Histone H1 is phosphorylated in non-replicating and infective forms of *Trypanosoma cruzi*. *Mol. Biochem. Parasitol*. **119**:265-271.

Elias MCB, Faria M, Mortara RA, Motta MCM, de Souza W, Thiry M. Schenkman,S. 2002. Chromosome localization changes in the *Trypanosoma cruzi* interphase nucleus. *Euk. Cell.* **1**:944-953.

Elias MCQB, Porto RM, Faria M, Schenkman S. 2003. Distinct Mechanisms Operate to Control Stage specific and Cell cycle Dependent Gene Expression in *Trypanosoma cruzi* in: Molecular Mechanisms in the Pathogenesis of Chagas Disease (J.M. Kelly, Ed). Eureka.com. http://www.eurekah.com/chapter/637. ISBN: 978-0-306-47849-9.

Elias MCB, Vargas NS, Zingales B, Schenkman S. 2003. Organization of satellite DNA in the genome of *Trypanosoma cruzi*. *Mol. Biochem. Parasitol*. **129**:1-19.

Ejchel TF, Ramirez MI, Vargas NS, Azevedo EB, Elias MCB, Zingales B, Schenkman, S. 2003. The largest subunit of the RNA polymerase II of *Trypanosoma cruzi* lacks the repeats in the carboxy-terminal domain and is encoded by several genes. *Parasitol. International*. **52**:243-249.

da Cunha JPC, Nakayasu ES, Elias MC, Pimenta DC, Tellez-Inon MT, Rojas F, Manuel M, Almeida IC, Schenkman S. 2005. *Trypanosoma cruzi* histone H1 is phosphorylated in a typical cyclin dependent kinase site accordingly to the cell cycle. *Mol. Biochem. Parasitol.* **140**:75-86.

Sérgio SCHENKMAN

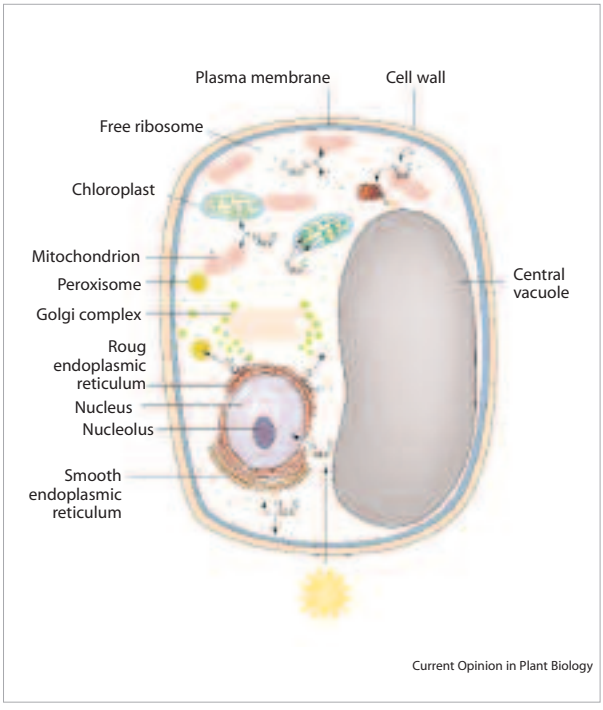
Universidade Federal de São Paulo (Unifesp)
Departamento de Microbiologia Imunologia e Parasitologia
R. Botucatu, 862, 8ª – Vila Clementino
04023-062 – São Paulo, SP – Brasil

+55-11-5575-1996 ext. 21
sschenkman@unifesp.br

DECIPHERING THE MOLECULAR MECHANISMS INVOLVED IN INTRACELLULAR PROTEIN TRAFFICKING IN PLANT CELLS

Márcio de Castro SILVA Filho

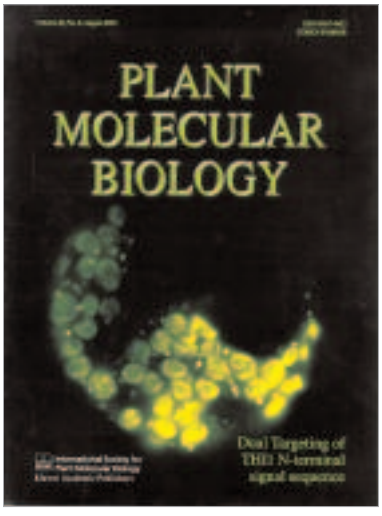
Luiz de Queiroz Higher School of Agriculture / University of São Paulo (USP)



One of our main objectives is to understand the molecular mechanisms responsible for the protein localization inside of the plant cells, especially the proteins directed to mitochondria and chloroplasts. In this proposal, it is intended to advance in the characterization of the regulatory mechanisms involved in the intracellular localization of different proteins, also integrating an evolutionary approach. Four sub-projects are presented. The first one aims to identify proteins interacting with THI1 protein of *Arabidopsis thaliana* on thiamin biosynthesis, by using the yeast double-hybrid technique. Moreover, it is intended to identify proteins responsible for the control of the double targeting of this protein, since it was shown by this group that this protein is directed simultaneously to mitochondria and chloroplasts by a post-transcriptional mechanism. The second sub-project aims to study in details the thiamin biosynthesis in plants. Preliminary studies carried through by this group involving techniques of in-silico analysis and molecular phylogenetics suggest that biosynthesis of thiamin in plants can involve more than one subcellular compartment, opposing previous works that suggest the occurrence of the pathway only in the plastids. In addition to a deep in-silico analysis there experiments of functional complementation in yeast cells of plant genes involved on thiamin biosynthesis will be carried out. Furthermore, the determination of the subcellular localization of all proteins involved in the process will be provided by gene fusions with the green fluorescent protein (GFP). The third project aims at to understand one of the most fascinating mechanisms of gene regulation recently described: riboswitches. The presence of structures in mRNA capable to interact directly with molecules intervening with its own translation has driven the attention of several groups worldwide. In the case of one of genes involved in thiamin biosynthesis, thic of *Arabidopsis thaliana*, it was shown that its mRNA presents an aptamer-like structure that allows its binding to either thiamin pirophosfate (TPP) or thiamin, regulating its expression. It is intended to verify experimentally if the occurrence of this structure intervenes with the expression of the thic gene as well as to the subcellular localization of the THIC protein. Moreover, a study will be carried through in silico in all genes whose products are involved in thiamin biosynthesis in order to identify the occurrence of aptamer-like structures and, in positive case, to verify its function experimentally. The fourth sub-project involves the characterization of the mechanism responsible for the localization of one metaloprotease of the thylakoid membrane, the FtsH-p1. Members of this family had been described as belonging to the Tat system (twin-arginine translocation system) for insertion in the thylakoid membrane. In a previous work of this group, it was shown that a member of this family does not present a classical RR motif responsible for its insertion in the membrane. Therefore, it is intended to characterize the mechanism responsible for the localization of protein, which suggests a distinct mechanism of those described in literature.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our group had shown that a protein named THI1 from *Arabidopsis thaliana* is encoded by a single gene and is subsequently translocated to mitochondria and chloroplasts by an alternative translation initiation mechanism based on two in-frame AUG start codons. When translation initiates in the first AUG the protein is translocated to plastids, whereas translocation from the second AUG, the protein is delivered into mitochondria. Previous examples of dual-targeting to mitochondria and chloroplasts involved ambiguous targeting sequences recognised by both sets of import machinery.



MAIN PUBLICATIONS

Ribeiro DT, Farias LP, Almeida JD, Kashiwabara PM, et al. 2005. Functional characterization of the *Thi*1 gene promoter region from *Arabidopsis thaliana*. *J. Exp. Bot.* **56**: 1797-1804.

Macasev D, Whelan J, Newbigin E, et al. 2004. Tom22′, an 8 kDa *trans*-site receptor in plants and protozoans, a conserved feature of the TOM complex that appeared early in the evolution of eukaryotes. *Mol. Biol. Evol.* **21**: 1557-1564.

Silva-Filho MC. 2003. One ticket for multiple destinations: dual targeting of proteins to distinct subcellular locations. *Curr. Opin. Plant. Biol.* **5**: 589-595.

Chabregas SM, Luche DD, Van Sluys M-A, et al. 2003. Differential usage of two in-frame translational start codons regulates subcellular localization of *Arabidopsis thaliana THI1*. *J. Cell. Sci.* **116**: 285-291.

Chabregas SM, Luche DD, Farias LP, et al. 2001. Dual targeting properties of the N-terminal signal sequence of *Arabidopsis thaliana* THI1 protein to mitochondria and chloroplasts. *Plant Mol. Biol.* **46**: 639-650. *cover article

Chaparro-Giraldo A, Barata RM, Chabregas SM, et al. 2000. Soybean leghemoglobin targeted to potato chloroplasts influences growth and development of transgenic plants. *Plant Cell Rep.* **19**: 961-965

Barata RM, Chaparro A, Chabregas SM, et al. 2000. Targeting of soybean leghemoglobin to tobacco chloroplasts: effects on aerobic metabolism in transgenic plants. *Plant Sci.* **155**: 193-202.

Silva-Filho MC. 1999. Translocation of a reporter protein into mitochondria mediated by a chloroplast transit peptide follows a normal import route. *J. Plant. Physiol.* **154**: 51-54.

Silva-Filho MC, Wieers M-C, Chaumont F, Flugge U-I, Boutry M. 1997. Different *in vitro* and *in vivo* targeting properties of the transit peptide of a chloroplast envelope inner membrane protein. *J. Biol. Chem.* **272**: 15264-15269.

Silva Filho MC, Chaumont F, Leterme S, Boutry M. 1996. Mitochondrial and chloroplast targeting sequences in tandem modify protein import specificity in plant organelles. *Plant Mol Biol*. **30**:769-780.

Márcio de Castro SILVA Filho

Escola Superior de Agricultura Luiz de Queiroz
Universidade de São Paulo (Esalq/USP)
Caixa Postal 83 – Departamento de Genética
Avenida Pádua Dias, 11 – Agronomia
CEP 13400-970 – Piracicaba, SP - Brasil

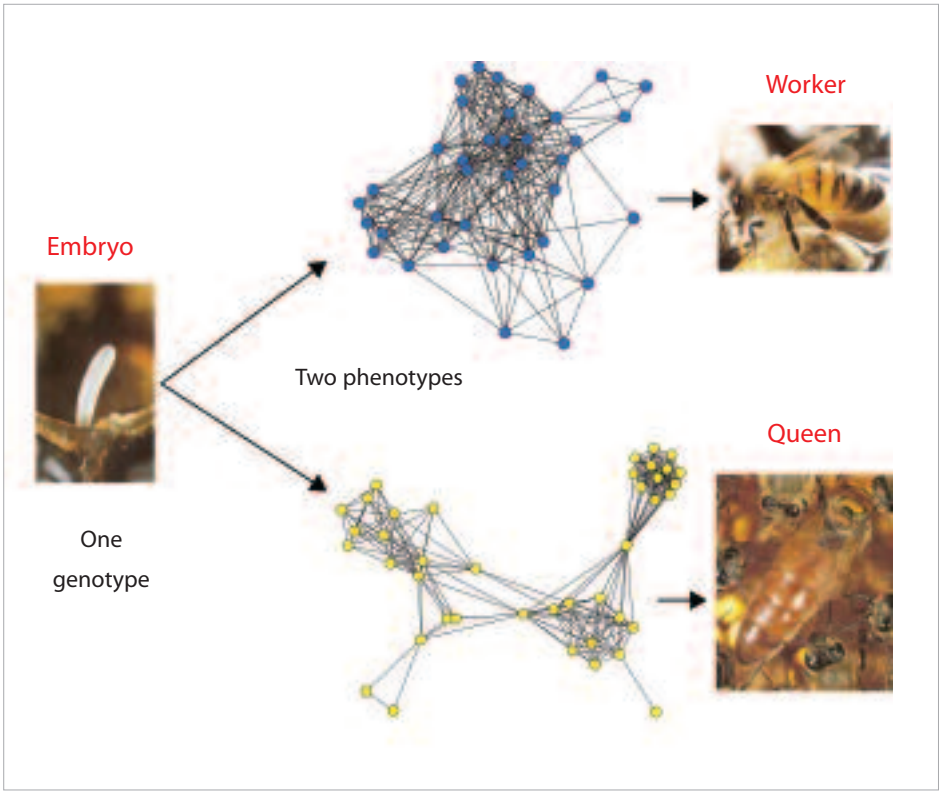
+55-19-3429-4442
mdcsilva@esalq.usp.br

Apis mellifera FUNCTIONAL GENOME – SEARCH FOR NEW GENES AND INTEGRATED NETWORK IN THE CONTEXT OF DEVELOPMENT, REPRODUCTION AND CASTE DIFFERENTIATION

Zilá Luz Paulino SIMÕES

Faculty of Philosophy, Sciences and Languages of Ribeirão Preto / University of São Paulo (USP)

With an active contribution (5021 ORESTES, deposited in the GenBank) and participation in the annotation of the recently sequenced genome of the honey bee our group now has consolidated knowledge and expertise in all aspects required for large-scale analyses of gene expression in honey bees. Following our long term questions of interest on mechanisms underlying caste and sex determination and those involved in reproduction and division of labor, already formulated in the pré-genomic era of honey bee research, we are now proposing a large-scale analysis of gene expression in different phases of the honey bee life cycle. We will focus on the following questions: the identification of genes involved in caste development, in programmed cell death of the larval ovary, in integument differentiation, in the activation of the adult ovary of queens and workers, in the functional cycle of the hypopharyngeal glands, and in early embryonic sex determination. In addition, we will screen our libraries for putative micro-RNAs to unveil the participation of this novel group of post-transcriptional regulators in the above contexts. The project adopts a dual strategy, a) the identification of candidate genes in the honey bee genome based on prior knowledge of gene function in other organisms, especially Gene Ontology attributes registered in flybase, and b) the search for novel genes by microarrays, subtractive hybridization strategies, and the generation of an embryonic cDNA library. The differential expression of all these genes will be tested and candidates of specific interest will be investigated by quantitative RT-PCR and silencing by RNAi. The joint information on gene expression in these contexts will be subjected to network analysis in order to detect functional linkage and organization in gene expression networks.



Networks depicting gene interaction based on the occurrence of overrepresented motifs in the Upstream Control Regions of Differentially Expressed Genes between A. mellifera *castes*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Investigating molecular underpinnings of honey bee caste development, differential reproduction and division of labor were perceived as core elements of our proposal. Our group had participated and coordinated the annotation of genes related to caste development and reproduction in the genome sequence project of *Apis mellifera*. The companion paper produced by our group comprised a bioinformatics analyses of genes specifically expressed in caste development. A subsequent expansion on this analysis on differential gene expression in queen versus worker development was based on new experimental data from micro array analyses and used a network approach to connect genes via their revealed putative upstream control region elements. These results paved the way for novel approaches to the study of developmental processes and their regulation in honey bees, for example the study of genes underlying the hormonally controlled expression of caste-specific morphological characters, such as the corbicula on the hind leg of workers and the massive autophagic cell death in the ovaries of worker larvae leading to the highly divergent ovary phenotypes observed in adult honey bee queens and workers. Further analyses along these lines are now being performed which will contribute to our understanding of queen/worker development by expression analyses of candidate genes or large scale differential gene expression screens. Such expression analyses, however, are only the first step towards understanding gene function, and in this direction our group has contributed to the field of honey bee functional genomics through the establishment of a successful RNAi approach, silencing gene function of the yolk protein vitellogenin. We focused on this gene, which is abundantly expressed in the female sex, both in queens and workers. There are novel functions postulated to vitellogenin, as a major regulator of longevity, which clearly is a major difference between queens and workers. In addition, vitellogenin was postulated to interact with juvenile hormone in a negative feedback circuitry to regulate the transition of a worker bee from within-hive tasks to foraging. Such aspects of functional genomics on major life history determinants were and are successfully being explored by our group in collaboration with Dr. Gro Amdam (Arizona State University) and Dr. Dolors Piulachs (Institut de Biologia Molecular de Barcelona, CID, CSIC).

MAIN PUBLICATIONS

The Honeybee Genome Sequencing Consortium. 2006. Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature*. **443**:931-949.

Barchuk AR, Cristino AL, Kucharski R, Costa LF, Simões ZLP, Maleszka R. 2007. Molecular determinants of caste differentiation in the highly eusocial honeybee *Apis mellifera*. *BMC Developmental Biology* 18. **7(1)**:70.

Cristino AL, Nunes FMF, Lobo CH, Bitondi MMG, et al. 2006. Caste development and reproduction: a genome-wide analysis of hallmarks of insect eusociality. *Insect Molecular Biology.* **15(5)**:703-714.

Bitondi MMG, Nascimento ADM, Cunha AD, Guidugli KR, Nunes FMF, Simões ZLP. 2006. Characterization and expression of the Hex 110 gene encoding a Glutamine-rich hexamerin in the honey bee, *Apis mellifera*. *Arch. Insect Biochem. Physiol*. **63**: 57-72.

Judice CC, Carazzole MP, Festa F, Sogayar MC, Hartfelder K, Pereira GAG. 2006. Gene expression profiles underlying alternative caste phenotypes in a highly eusocial bee, *Melipona quadrifasciata*. *Insect Molecular Biology.* **15**:33-44.

Cristino AS, Nascimento AM, Costa L da F, Simoes ZL. 2006. A comparative analysis of highly conserved sex-determining genes between *Apis mellifera* and *Drosophila melanogaster*. *Genet. Mol. Res*. **154**:68.

Amdam GV, Nilsen K-A, Norberg K, Fondrk MK, Hartfelder K. 2007. Variation in endocrine signaling underlies variation in social life-history. *American Naturalist.* **170**: 37-46.

Miranda MP, Simões ZLP, Bitondi MMG. 2007. A cuticle protein gene in the honey bee: expression during development and in relation to the ecdysteroid titer. *Insect Biochemistry and Molecular Biology.* (in press).

Bitondi MMG, Nascimento AM, Cunha AD, et al. 2006. Characterization and expression of the hex 110 gene encoding a glutamine-rich hexamerin in the honey bee, *Apis mellifera*. *Arch. Insect Biochem. Physiol*. **63**: 57-72.

dos Santos AM, Nascimento AM, Bitondi MMG, et al. 2008. Identification of a juvenile hormone esterase-like gene in the honey bee, *Apis mellifera L.* expression analysis and functional assays. *J. Comp. Physiol*. (in press).

Zilá Luz Paulino SIMÕES

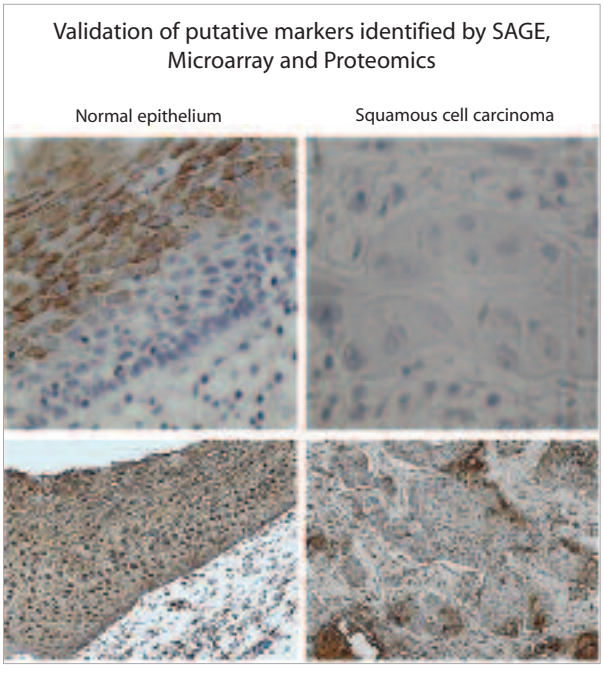
Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto / Universidade de São Paulo (USP)
Departamento de Biologia
Avenida Bandeirantes, 3900 – Monte Alegre
14040-901 – Ribeirão Preto, SP – Brasil

+55-16-602-4332
zlpsimoe@usp.br

MARKERS OF AGGRESSIVE BEHAVIOUR IN HEAD AND NECK TUMORS

Eloiza Helena TAJARA da Silva

São José do Rio Preto Medicine School



Above, KRT4 low expression in oral cell carcinomas. Below, ANXA1 low expression in larygeal squamous cell carcinomas

Head and neck squamous cell carcinoma is one of the most frequent neoplasias in Brazil. Patients in early stages frequently exhibit few symptoms, resulting in diagnosis delay and decrease in survival. Also, clinically and histologically similar lesions can follow significantly different clinical courses and show different responses to therapy. Unfortunately, despite intense research and improvements in early detection and therapeutic strategies, the prediction of tumor behavior in this group of neoplasias is still limited and the 5-year survival rate remains low.

The present study aims to investigate molecular markers of tumor aggressiveness in head and neck carcinomas that may be relevant for prognosis and therapeutic strategies. Specific aims include: (1) combining genomic and proteomic techniques, to investigate differences in gene and protein expression profiles from head and neck carcinomas and apparently normal counterparts (2) and their relations to clinical and laboratory parameters (disease stages or evolution); (3) to investigate genetic polymorphisms associated with metabolic pathways which are involved in tumor progression; (4) to validate emerging molecular markers by quantitative PCR, immunohistochemistry and genotyping in a large number of specimens.

To achieve these aims, samples collected by the team of project will be analyzed. This series includes clinically well-characterized tumor samples, surgical margins and blood samples from 1,547 patients and blood samples from 554 controls.

The data may elucidate the mechanisms governing genetic changes during tumor progression and their relation to the tissue genetic background, resulting in new insights into signalling and metabolic pathway abnormalities that could be useful therapeutic targets.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Combining genomic and proteomic techniques, differences in gene and protein expression profiles from HNSCC and apparently normal counterparts and their relations to clinical and laboratory parameters were investigated as well as genetic polymorphisms associated with metabolic pathways involved in tumor progression. Epidemiological analysis according to demographical variables and risk factors showed a higher average age in cases than controls. Most patients were males, Caucasian and smokers and more than 70% are alive 20 months after the diagnosis. Oral cavity and larynx were the most frequent sites of tumor, the latter showing the highest survival rate. There were statistical significant differences between cases and controls for all variables studied, indicating possible environmental factors to be explored in their relation with HNSCC in future analysis.

The analysis of gene expression by SAGE methodology identified subsets of differentially expressed tags between laryngeal carcinomas and normal tissues, and between metastatic and non-metastatic samples. Differential expression of a subset of genes was confirmed by quantitative polymerase chain reaction (qPCR). The product of one of these genes, ANXA1, was also investigated at the subcellular level by immunohistochemical analysis and the results showed down-regulation in dysplastic, tumoral and metastatic lesions, providing evidence for the progressive migration of ANXA1 from the nucleus towards the membrane along the laryngeal tumorigenesis.

A microarray analysis was also performed in oral squamous cell carcinomas and identified punctual differences between subsites and TNM classified samples, suggesting that oral tumors may respond differently to therapies currently under development.

Still analyzing data of HNSCC transcriptome, more than four hundred splicing events were evaluated. A subset of 43 new splicing isoforms was validated by qPCR and DNA sequencing and differences were detected between normal and metastatic samples. With respect to polymorphisms, the DNA of >250 samples was investigated for 45 single nucleotide polymorphisms. Differences of genotype frequencies between aggressive and less-aggressive oral tumors could be detected for a number of SNPs. The most significant were located on the genes *Mmp*7, *Mmp*14, *Gstp*1 and *Cdkn*1*A*. The proteomic analysis explored cancer-related proteins in HNSCC. Qualitative and quantitative variations in aggressive and less-aggressive tumors were identified by bidimensional electrophoresis and mass spectrometry, including cytokeratins, heat shock proteins and proteins involved in cell signaling, adhesion, transport, and apoptosis, indicating their potential as cancer-related markers.

One of the hallmarks of the Head and Neck Genome Project is the close interaction among the groups that perform different analyses. The continuous update and exchange of results enable a more comprehensive view of the results. By using this approach, consistent data on gene and protein expression and SNP frequencies related to aggressive and non-aggressive tumors may be achieved and the data used for predicting outcome and rational targets in HNSCC.

MAIN PUBLICATIONS

de Marqui AB et al. 2006. Solubilization of proteins from human lymph node tissue and two-dimensional gel storage. *J. Biochem Mol Biol.* **39(2)**:216-22.

Leopoldino AM et al. 2006. Expression, purification, and circular dichroism analysis of human CDK9. *Protein Expr Purif.* **47(2)**:614-20.

Rodrigues-Lisoni FC et al. 2006. Annexin A1: from gene organization to physiology. *Calcium Binding Proteins* **1(2)**:72-76.

Rodrigues-Lisoni FC et al. 2006. *In vitro* and *in vivo* studies on CCR10 regulation by Annexin A1. *FEBS Lett*. **580(5)**:1431-8.

Tajara EH, Dias-Neto E, Silva Jr WA, et al. 2006. Head and Neck Genome Project. Markers of aggressive behaviour in head and neck tumors. *Oral Oncology.* **11**:3-8.

Silistino-Souza R et al. 2007. Annexin 1: differential expression in tumor and mast cells in human larynx cancer. *International J. Cancer*. **120(12)**:2582-9.

Stabenow et al. 2007. Metástase linfática cervical no carcinoma espinocelularde vias aerodigestivas superiores: análise crítica de fatores preditivos, pelo método de regressão logística. *Rev. Brasil. Cir. Cabeça e Pescoço.* **36(4)**:202-208.

Alves et al. Annexin 1 Subcellular Expression in Laryngeal Squamous Cell Carcinoma. *Histopathology,* submitted.

Severino et al. Global gene expression profiling of oral squamous cell carcinoma reveals great molecular heterogeneity within TNM classes and molecular signatures associated with tumor location. *BMC Cancer*, submitted.

Silveira, Varuzza et al. Using statistical and bioinformatics approaches for identification of molecular markers in sage libraries from head and neck carcinomas. *BMC Genomics*, submitted.

Eloiza Helena TAJARA da Silva

Faculdade de Medicina de São José do Rio Preto
Avenida Brigadeiro Faria Lima, 5416 – Vila São Pedro
CEP 15090-000 – São José do Rio Preto, SP – Brasil

+55-17-3201-5737 ext. 5809
tajara@famerp.br

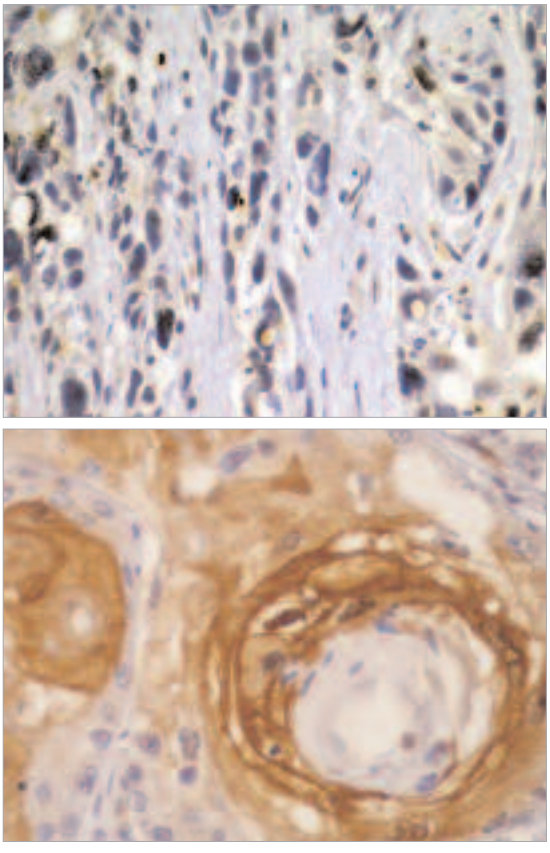
IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF MOLECULAR MARKERS INVOLVED IN HEAD AND NECK TUMOR FORMATION BY THE ANALYSIS OF THE PATTERN OF DIFFERENTIAL METHYLATION

Sandro Roberto VALENTINI

School of Pharmaceutical Sciences / Paulista State University (Unesp)

IMMUNOHISTOCHEMISTRY OF CRABP2

Squamous Cell Carcinoma of Pharynx



Above, not detected in poorly differentiated tumor. And below, detected in well differentiated tumor

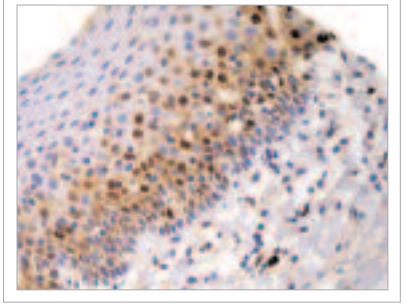
DNA methylation of the cytosine of CpG dinucleotides frequently leads to transcriptional silencing and is involved in several normal physiological conditions. However, aberrant DNA methylation is highly implicated in cancer biology, repressing the expression of genes associated with tumor formation and progression. Therefore, the analysis of the methylation pattern of CpG islands in tumors can reveal molecular markers useful for diagnosis and/or prognosis of neoplasia and lead to the identification of new therapeutic targets. The main goal of this project is to identify genes silenced in head and neck tumors by DNA methylation in their regulatory regions, which can be used as molecular markers for patients with this type of tumor. The specific aims are: (a) identify methylated genes in head and neck carcinoma cell lines, after reactivation of gene expression with a demethylation drug, by using RaSH – Rapid Subtraction Hybridization; (b) validate by micro array the gene expression reactivation by the demethylating drug in head and neck carcinoma cell lines; (c) confirm the gene expression reactivation of the selected genes by qRT-PCR in the cell lines; (d) analyze the methylation pattern of CpG islands of the selected genes in the cell lines, by DNA conversion with sodium bisulfite followed by DNA sequencing; (e) investigate the differences in the methylation patterns of the selected genes between head and neck carcinomas and normal tissues, using MSP – Methylation Specific PCR; (f) analyze the expression of the selected methylated genes in head and neck carcinomas by immunohistochemistry and correlate the results with clinical data of patients and (g) functionally characterize the genes methylated in head and neck tumors by using the yeast *Saccharomyces cerevisiae* as a model organism (for genes with homologs in yeast) or the yeast two-hybrid system (for genes without homology). In parallel, investigate the methylation pattern of LHX6 and ADAM23 in this type of tumor.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The combination of RaSH and microarray resulted in the identification of 78 genes reactivated by treatment with the demethylating agent 5-aza-2'-deoxycytidine (5-azadC) in head and neck carcinoma cell lines from different anatomic sites (pharynx, mouth floor, supraglottis and tonsils). The genes containing bonafide CpG islands in their regulatory regions and which were reactivated in at least 2 out of the 4 carcinoma cell lines used were selected for further validation by qRT-PCR. Out of the 36 genes analyzed, reactivation of gene expression was confirmed for 5 genes (*Crabp*2, *Mx*1, *Slc*15*A*3, *Capza*1 and *Ktn*1). So far, bisulfite DNA conversion assay followed by nucleotide sequencing has confirmed differential DNA methylation of the genes *Crabp*2, *Mx*1 and *Slc*15*A*3 in at least one of the head and neck carcinoma cell lines. Concerning *Crabp*2, MSP assays performed with a panel of 120 samples of head and neck carcinomas demonstrated a high frequency (65%) of DNA methylation in this gene. Protein expression of CRABP2 was then analyzed by immunohistochemistry using a Tissue Micro array of 73 tumor samples. Accordingly, it was shown in most of the cases (62%), that the head and neck carcinoma samples are negative (11%) or weakly positive (51%) for the CRABP2 protein, confirming the data obtained by MSP. Next, the expression pattern of the CRABP2 protein was compared with the global survival rates of patients. This analysis showed a statistically significant correlation between lack of the CRABP2 protein and lower survival rates of patients. Therefore, the data obtained so far have revealed that the presence of methylation in the regulatory region of *Crabp*2 that results in its silencing is a marker of poor prognosis for patients with head and neck carcinoma tumors.

IMMUNOHISTOCHEMISTRY OF CRABP2

Normal Epithelial Tissue



Detected only in the basal layer

In the parallel study, with the genes *Lhx*6 e *Adam*23, interesting results were obtained. For *Lhx*6, it was demonstrated that the methylation pattern of the *Dime*-6 fragment is correlated with gene silencing of the shorter isoform of *Lhx*6, suggesting that hypermethylation of *Dime*-6 is a good tumor marker for head and neck cancer. Considering *Adam*23, a correlation was found between DNA methylation and the stage of the tumors analyzed.

MAIN PUBLICATIONS

Estecio MRH, Youddrg EM, Rahal P, Fukuyama EE, Góis-Filho JF, Maniglia JV, Goloni-Bertollo EM, Issa J-PJ, Tajara EH. 2006. LHX6 is a sensitive methylation marker in head and neck carcinomas. *Oncogene*. **25**: 5018-26.

Calmon MF, Colombo J, Carvalho F, Souza FP, Filho JFG, Fukuyama EE, Camargo AA, Caballero OLS, Tajara EH, Cordeiro JA, Rahal P. 2007. Methylation profile of genes *Cdkn2A* (p14 and p16), *Dapk*1, *Cdh*1, and *Adam*23 in head and neck cancer. *Cancer Genetics and Cytogenetics*. **173**: 31-1.

Calmo MF, Rodrigues RV, Kaneto CM, Moura RP, Silva SD, Mota LDC, Pinheiro DG, Torres C, de Carvalho AF, Cury PM, Nunes FD, Soares FA, da Silva AMA, Kowalski LP, Brentani H, Zanelli CF, Silva Jr. WA, Rahal P, Tajara EH, Carraro DM, Camargo AA, Valentini SR. Epigenetic reactivation by DNA demethylation identified new molecular markers in head and neck tumors. *Cancer Research*, under submission.

Sandro Roberto VALENTINI

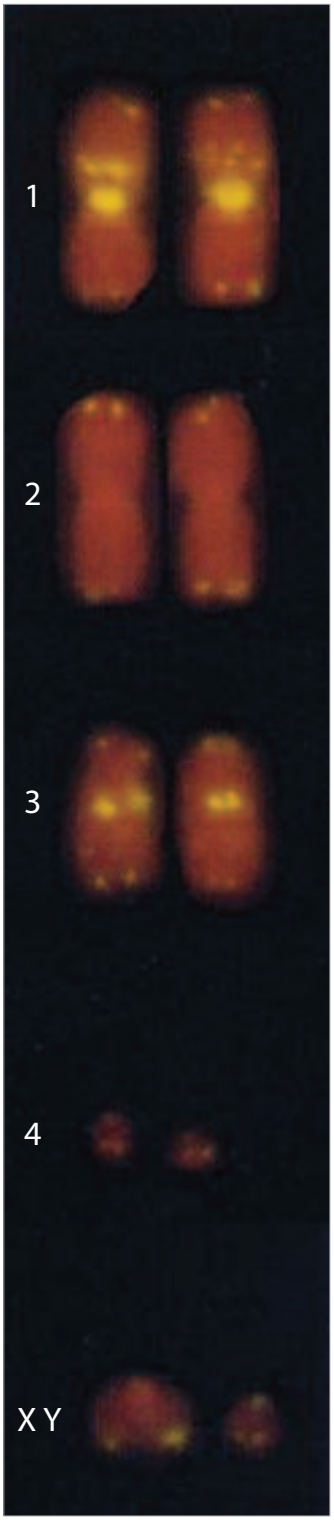
Faculdade de Ciências Farmacêuticas de Araraquara
Universidade Estadual Paulista (Unesp)
Departamento de Ciências Biológicas
Rodovia Araraquara-Jaú, Km01
CEP 14801902 – Araraquara, SP – Brasil

+55-16-3301-6954
valentsr@fcfar.unesp.br

EVOLUTIONARY STUDIES IN VERTEBRATES: I. CYTOGENETIC, MOLECULAR AND MORPHOLOGICAL ANALYSES. II. KARYOTYPICAL SURVEY OF BRAZILIAN FAUNA. III. CELL AND TISSUE COLLECTION OF MAMMALS, LIZARDS AND AMPHIBIANS

Yatiyo Yonenaga YASSUDA

Institute of Biosciences / University of São Paulo (USP)



Karyotype of Akodon sp., *male, 2n=10, after fluorescent* in situ *hybridization (FISH) with telomeric probes*

The present project has been conducted under a multidisciplinary perspective and it aims the study of Brazilian biodiversity, including rodents, marsupials, lizards and amphibians on the basis of cytogenetic, molecular and morphological data.

Chromosomal studies have been carried out based on conventional (Giemsa and Ag-NOR-staining; C-, G-, R-bands) and molecular cytogenetics (fluorescence *in situ* hybridization or FISH) using telomeric and/or ribosomal probes, and also ZOO-FISH with probes generated from specific micro dissected chromosomes. This approach has achieved solutions for a number of cases in different groups of vertebrates. In lizards and amphibians, by instance, differential chromosome staining still represents important information to be reported and these data have contributed for characterization of populations and species, and for a better understanding of the chromosomal and evolutionary processes that groups have undergone.

Regarding morphologic issues of *Akodon*, it has been evinced which traits are the most relevant ones to be considered as informative characters for the genera. Furthermore, the chromosomal polymorphism and the range achieved for each karyomorph (2n=14, 15, 16) of *A. cursor* throughout the Atlantic rainforest have been informative in order to have the distribution of the species mapped.

DNA sequences from mitochondrial and nuclear also represent an important tool that we are using to recover phylogeny of amphibians, reptiles and mammals. These data associated with geographic distribution, cytogenetic and morphological information of the species have helped to reconstruct the evolutionary history of each different group.

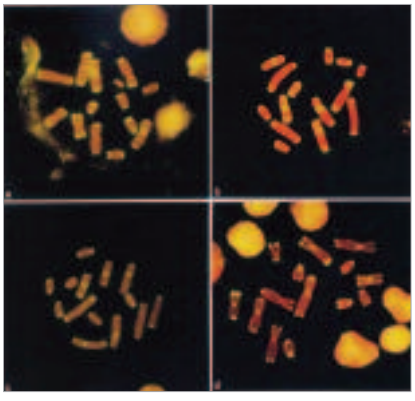
We have also been involved in fauna surveys and fauna rescues in hydroelectric power plants which occurred in different Brazilian biomes, e.g. Amazonian and Atlantic forests, Cerrado, Caatinga, etc., and the results revealed a number of unknown species within rodents, marsupials, lizards and amphibians.

Our laboratory also stores tissues and cells obtained from fibroblast cultures in a "Collection of Cells and Tissues of mammals, reptiles and amphibians", which represents a valuable stock for rare, endangered and ordinary species collected in the Brazilian territory for about the last 20 years, as a result of our collaboration with Dr. Miguel T. Rodrigues (Zoology Department, Biosciences Institute, USP-SP) and Dr. Mario de Vivo (Zoology Museum, USP-SP), both projects supported by FAPESP.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In our project, cytogenetical analyses have already performed in species of 25 rodents, 13 marsupials, six bats, 32 lizards and 16 amphibians, most of them of unknown or misidentified species, which were characterized on the basis of karyotypic data.

A population level study in the rodent *Akodon cursor* has been conducted by different authors, since the description of the species in 1972 in our laboratory, with about 600 specimens sampled, in order to estimate the frequencies of the chromosomal polymorphism (2n=14, 15 e 16) within this species.



Intraspecific variation of pericentromeric signals after fluorescent in situ *hybridization (FISH) with telomeric probes in the marsupial* Micoureus demerarae *(2n=14). a) Two interstitial signals. b) Four. c) Five. d) Six*

Presently, we are analyzing sequences from mitochondrial and nuclear regions of *Akodon* within a phylogenetic perspective. In 1998, a karyotype of an unknown species of *Akodon* with 2n=9 and 10, the lower diploid number among rodents, was described by us. In 2006, a study about the phylogenetic relationships and karyotype evolution of this new *Akodon* and another related species was published, on the basis of sequences of the cytochrome b gene and chromosomes. It is underway the karyotypic description and comments on morphology of the rodent *Callistomys pictus*, an endemic and threatened species from State of Bahia, Brazil.

In the marsupial *Micoureus demerarae* (2n=14), we detected an intraspecific variation in the distribution of interstitial telomeric sequences, after fluorescent *in situ* hybridization.

Several species of lizards have been karyotyped, and chromosomal mechanisms of sex determination of the XX:XY type and multiple ones, and also an extensive karyotypic variability in the family Gymnophthalmidae, have been detected by our group. Within a molecular perspective, a study assembling DNA sequences for 26 genera of gymnophthalmids resulted in a new classification for the family was proposed.

The amazing diversity of Brazilian amphibians with problematic issues relative to systematics, and threaten to several species in face of environmental degradation, makes this group very suitable for biological studies. We are studying this group under cytogenetical, molecular and morphological perspectives.

The multidisciplinary studies we have conducted have contributed to a better understanding of the evolutionary processes responsible for the differentiation and maintenance of vertebrate species of the enormous Neotropical biodiversity.

MAIN PUBLICATIONS

Amaro-Ghilardi RC, Rodrigues MT, Yonenaga-Yassuda Y. 2007. Chromosomal studies in four species of genus *Chaunus* (Bufonidae, Anura): Localization of telomeric and ribosomal sequences after fluorescent *in situ* hybridization (FISH). *Genetica*. (DOI: 10.1007/s10709-007-9218-6).

Bertolotto CEV, Pellegrino KCM, Yonenaga-Yassuda Y. 2004. Ocurrence of B chromosomes in lizards: a review. *Cytogenetics and Genome Research.* **106**: 243-246.

Fagundes V, Christoff AU, Scalzi-Martin JM, Moreira-Filho CA, Yonenaga-Yassuda YX. 2000. Y translocation revealed by chromosome microdissection and FISH in fertile XY in the Brazilian rodent *Akodon montensis*. *Cytogenetics and Cell Genetics.* **88**:124-129.

Fagundes V, Camacho JPM, Yonenaga-Yassuda Y. 2004. Are the dot-like chromosomes in *Trinomys iheringi* (Rodentia, Echimyidae) B chromosomes? *Cytogenetics and Genome Research*. **106**:159-164.

Pagnozzi JM, Silva MJJ, Yonenaga-YassudaY. 2000. Intraspecific variation in the distribution of the interstitial telomeric (TTAGGG)n sequences in *Micoureus demerarae* (Marsupialia: Didelphidae). *Chromosome Research.* **8**: 585-591.

Pellegrino KCM, Rodrigues MT, Yonenaga-Yassuda Y. Sites Jr. JW. 2001. A molecular perspective on the evolution of microteid lizards (Squamata, Gymnophthalmidae), and a new classification for the family. *Biological Journal of Linnean Society.* **74**: 315-338.

Silva MJJ, Yonenaga-Yassuda Y. 2004. B chromosomes in Brazilian rodents. *Cytogenetics and Genome Research.* **106**: 257-263.

Silva MJJ, Patton JL, Yonenaga-Yassuda Y. 2006. Phylogenetic relationships and karyotype evolution in the sigmodontinae rodent *Akodon* (2n= 10 and 2n= 16) from Brazil. *Gen. and Mol. Biology.* 29: 469-474.

Ventura K, Silva MJJ, Fagundes V, Christoff AU, Yonenaga-Yassuda Y. 2006. Non-telomeric sites as evidence of chromosomal rearrangement and repetitive (TTAGGG)n arrays in the heterochromatin and euchromatic regions in four species of *Akodon* (Rodentia, Muridae). *Cytogenetics and Genome Research* **115**: 169-175.

Yatiyo Yonenaga YASSUDA

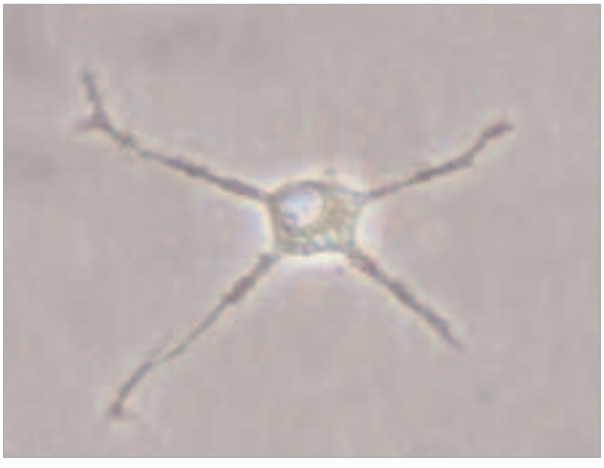
Instituto de Biociências
Universidade de São Paulo (USP)
Rua do Matão, 277 – Butantã
CEP 05508-900 – São Paulo, SP – Brasil

+55-11-3091-7574 r. 7574
yyassuda@usp.br

FUNCTIONAL MODULATION OF DENDRITIC CELLS IN DIFFERENT PATHOPHYSIOLOGICAL CONDITIONS

José Alexandre Marzagão BARBUTO

Institute of Biomedical Sciences / University of São Paulo (USP)



A dendritic cell (DC)

Dendritic Cells (DCs) are the major antigen presenting cells and the possibility of their generation *in vitro* has renewed the interest in immunotherapy protocols. However, their study shows that they are functionally heterogeneous and under poorly defined control mechanisms. The unravelling of such mechanisms may allow DCs exploitation in more efficient immunotherapy protocols. Therefore, the aim of the present project is the study of human DCs differentiation *in vitro*. In seven sub-projects, evaluating their function and membrane phenotype, under the effect of periodontal bacteria, different temperatures, apoptotic cells, heat-shock proteins, tumor cells, jaundice, hormones (specifically melatonin), and immunomodulatory drugs (specifically cyclophosphamide).

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Dendritic cells (DCs) have a unique role in the immune system due to their ability to connect innate mechanisms of danger recognition to acquired immune responses. They do so by capturing and processing antigens at the same time they recognize disturbances in their environment. This latter recognition induces physiological changes that allow DCs to convey, to the T lymphocytes, signals inducing either "effector" responses or tolerance.

Having such a key role in determining the fate of the immune responses induced by contact with antigens, it is not unexpected that situations that lead to disease may be associated with disturbances of DC physiology, and their characterization, in various conditions, has been the goal of this project. We therefore have been studying DC phenotype and function, *in vivo* and *in vitro*, in situations that go from psychological stress to metastatic cancer in human patients. Not surprisingly, DCs are affected by such conditions in ways that are coherent with clinical observations, but, furthermore, also implicate DCs in the pathogenesis of diseases that go from infections to autoimmunity and cancer.

A clearer understanding of this involvement will allow the improvement of DC-based therapeutic interventions, like those we have just started, in patients with various cancer types (neuro and glioblastoma, osteosarcoma, and lymphomas). Also with that goal, we have been attempting to improve antigen presentation by DCs, through transfection of tumor-derived RNA into antigen-presenting cells.

Considering external influences, until now we have noted that psychological status, environment temperature and pH, melatonin, presence of cancer (*in vitro* and *in vivo*) affect DC phenotype and function, decreasing or modulating their lymphostimulatory activity and favoring the response of different T cell populations and/or the secretion of different cytokine patterns. The mechanisms involved in the genesis of these phenomena are under investigation and their unraveling will allow the understating of DC role in the pathogenesis of many of these diseases and the design of more effective therapeutic interventions.

MAIN PUBLICATIONS

Reis ES, Barbuto JA, Köhl J, Isaac L. 2008. Impaired dendritic cell differentiation and maturation in the absence of C3. *Mol Immunol.* Apr **45(7)**:1952-62. Epub. 2007 Dec 3.

Baleeiro RB, Anselmo LB, Soares FA, Pinto CA, et al. 2008. High frequency of immature dendritic cells and altered in situ production of interleukin-4 and tumor necrosis factor-alpha in lung cancer. *Cancer Immunol Immunother.* 2008 Feb 20. [Epub ahead of print]

Baleeiro RB, Bergami-Santos PC, Tomiyoshi MY, et al. 2008. Expression of a dendritic cell maturation marker CD83 on tumor cells from lung cancer patients and several human tumor cell lines: is there a biological meaning behind it? *Cancer Immunol Immunother.* Feb **57(2)**:265-70. Epub 2007 Jul 13.

Reis ES, Barbuto JA, Isaac L. 2007. Complement components, regulators and receptors are produced by human monocyte-derived dendritic cells. *Immunobiology*. **212(3)**:151-7. Epub 2007 Jan 3.

Antunes AA, Dall'Oglio MF, Barbuto JA, Leite KR, Srougi M. 2006. Pure sarcomatous recurrence of clear cell renal carcinoma following radical nephrectomy and dendritic cell vaccination. *São Paulo Med J.* **124(3)**:161-2.

Reis ES, Barbuto JA, Isaac L. 2006. Human monocyte-derived dendritic cells are a source of several complement proteins. *Inflamm Res.* **55(5)**:179-84.

Treglia-Dal Lago M, Jukemura J, Machado MC, da Cunha JE, Barbuto JA. 2006. Phagocytosis and production of H2O2 by human peripheral blood mononuclear cells from patients with obstructive jaundice. *Pancreatology*. **6(4)**:273-8. Epub 2006 Apr 19.

Anselmo LB, Gross JL, Haddad F, Deheinzelin D, Younes RN, Barbuto JA. 2005. Functional analysis of cells obtained from bronchoalveolar lavage fluid (BALF) of lung cancer patients. *Life Sci*. **76(25)**:2945-51.

Neves AR, Ensina LF, Anselmo LB, Leite KR, et al. 2005. Dendritic cells derived from metastatic cancer patients vaccinated with allogeneic dendritic cell-autologous tumor cell hybrids express more CD86 and induce higher levels of interferon-gamma in mixed lymphocyte reactions. *Cancer Immunol Immunother*. **54(1)**:61-6.

José Alexandre Marzagão BARBUTO

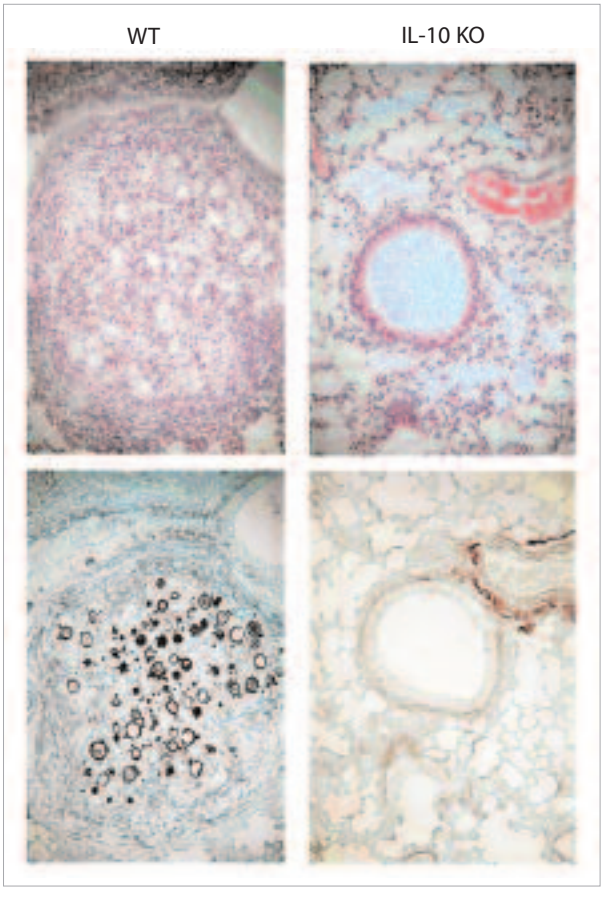
Instituto de Ciências Biomédicas
Universidade de São Paulo (USP)
Avenida Professor Lineu Prestes, 1730, N134
Cidade Universitária
05508-000 – São Paulo, SP – Brasil

+55-11-3091-7375
jbarbuto@icb.usp.br

ACTIVATION OF THE IMMUNE SYSTEM IN A PULMONARY MODEL OF FUNGAL INFECTION (PARACOCCIDIOIDOMYCOSIS). EFFECT OF FUNGAL AND HOST FACTORS IN THE SEVERITY OF THE DISEASE

Vera Lúcia Garcia CALICH

Institute of Biomedical Sciences / University of São Paulo (USP)



Genetic deficiency of IL-10 (IL-10 KO mice, right micrographs) leads to microbiological cure of P. brasiliensis *pulmonary infection (WT control mice, left micrographs). Upper, H&E; lower, Groccot stained lesions (100x)*

Paracoccidioidomycosis (PCM) is the most prevalent deep mycosis in Latin America and presents a wide spectrum of clinical and immunological manifestations. Our group established a genetically controlled murine model of PCM, where A/Sn mice develop an infection which mimics the benign disease (immune responses which favor cellular immunity) and B10.A animals present the progressive disseminated form of PCM (preferential activation of B cells and impairment of cellular immune responses). This pattern of immunological reactivity led us to postulate that the Th1/Th2 paradigm of immune response could be applied to explain the resistant/susceptible patterns in experimental PCM. Cytokines studies, mainly in the pulmonary model of infection, have confirmed that production of IFN-Y, TNF-a and IL-12 are linked with resistance but more complex immunological mechanisms, not Th1/Th2 mediated, are associated with genetic susceptibility to *P. brasiliensis* infection. We are now proposing further studies in our experimental pulmomary model of PCM. We intend to expand our studies on the influence of some cells and mediators of innate immunity (macrophages, NK cells, nitric oxide, IL-10, leucotrienes, *P. brasiliensis* lipids, TLR-4, chemokines) in the adaptative immunity and severity of pulmonary PCM. We also intend to further explore the role of TDC8 and TCD4 lymphocytes in the immunoprotection against *P. brasiliensis* infection. Several approaches, genetic strains of mice as well as immunomanipulations will be used to reach a better comprehension of the immunoprotective mechanisms operating in pulmonary PCM. After i.t. infection with on million yeast cells, mice will be studied regarding the severity of infection in lungs, liver and spleen, production of specific isotypes, delayed hypersensitivity reactions, levels of pulmonary and hepatic cytokines and organs histopathology. In addition, studies on the cellular composition of bronchoalveolar lavage fluids and lung infiltrating lymphocytes will be performed. When required, the activation of these cells will be evaluated by the expression of adhesion and co-stimulatory molecules besides the characterization of its ability to secrete pro- and anti-inflammatory mediators.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The main purpose of this project is to study some mechanisms of innate immunity which interfere with the protective or deleterious adaptative immune responses that further develop in a pulmonary model of fungal infection. Our studies on the function of CD4+ and CD8+ T cells in the immunity developed by susceptible, intermediate and resistant mice after pulmonary infection with *P. brasiliensis* demonstrated that: a) unexpectedly, fungal loads are mainly controlled by CD8α + T cells; b) genetic susceptibility of hosts appears to be associated with deletion or anergy of CD4+ T cells, and finally, d) a balanced type1/type2 immunity is associated with genetic resistance to *P. brasiliensis* infection. Our results on the role of TLRs in paracoccidioidomycosis suggest *P. brasiliensis* yeasts use TLR2 and TLR4 to gain entry into macrophages and infect mammalian hosts. Indeed, *P. brasiliensis* yeasts appear to be recognized by TLR2 and TLR4, resulting in increased phagocyte ability, NO secretion and fungal infection of macrophages. Thus, interaction with TLRs could be considered a pathogenicity mechanism of *P. brasiliensis*. We could also verify that alveolar macrophages of susceptible mice are very reactive to *P.brasiliensis* components and pro-inflammatory mediators are secreted by cells involved in the innate immunity of lungs. The excessive production of NO, however, inhibits the initial development of CD4+ T-cell-immunity by active induction of T cell anergy or deletion. The elevated expression of co stimulatory molecules (MHC class I, CD40, CD80) by APC can directly activate CD8+ T cells without the help of CD4+ T lymphocytes. This pattern of immunity can explain the efficient mechanism of innate immunity resulting, however, in poor T cell mediated immunity. Alveolar macrophages from resistant mice respond to *P.brasiliensis* infection by secreting low amounts of IL-12, but high levels of TGF-β and TNF-α. This explains the inefficient natural immunity of resistant mice. The low levels of NO production, however does not impair T cell immunity. So, resistant animals slowly develop *P. brasiliensis* specific CD4+ and CD8+ T lymphocytes, which control fungal growth and organize lesion morphology. This model does not exclude the previously proposed Th1/Th2 model of *P. brasiliensis* control, but explains why the excessive activation of the immune system can result in enhanced susceptibility to the fungus and severe pathology.

MAIN PUBLICATIONS

Calich VLG, Reis GA, Ribeiro-Gomes FL, A, MMC, P, N M, Borges, VM, M, BM. 2005. Fas ligand mediated turnover of neutrophils drives infection by an intracellular pathogen. *J. Infect. Dis.* USA, **192**: 1128-1134.

Calich VLG, Pina A, Cano LE, Saldiva PHN. 2006. Neutrophil role in pulmonary paracoccidioidomycosis depends on the genetic pattern of hosts. *J. Leukoc. Biol*. USA, v.79, n.4, 1202-1213.

Calich VLG, Blotta MHSL. 2005. Pulmonary Paracoccidioidomycosis. In: *Fungal Immunology, From an Organ Perspective*. Fidel, P. L & Huffnagle, G. B. Eds, Springer, New York, NY, pp. 201-228.

Arruda C, Kashino SS, Fazioli RA, Calich VLG. 2007. A primary subcutaneous infection with *Paracoccidioides brasiliensis* leads to immunoprotection or exacerbated disease depending on the route of challenge. *Microbes & Infec*. **9**:308-316.

Arruda C, Vaz CAC, Calich VLG. 2007. Aseptic cure of pulmonary paracoccidioidomycosis can be achieved after a previous subcutaneous immunization of susceptible but not resistant mice. *Microbes & Infec.* **9**:701-713.

Calich VLG, Pina A. 2007. Response to "Unfair to ignore long established line of published research". *J. Leuk. Biol.* **81**: 858-859.

Chiarella AP, Arruda C, Pina A, Costa TA, Valente-Ferreira RC, Calich VLG. 2007. The relative importance of CD4+ and CD8+ T cells in immunity to pulmonary paracoccidioidomycosis. *Microbes & Infect.* **9**:1078-1088.

Calich VLG, Costa TA, Felonato M, Arruda C, Bernardino S, Loures FV, Ribeiro LRR, Valente-Ferreira RC, Pina A. 2008. Innate Immunity to *Paracoccidioides brasiliensis* infection. *Mycopathol*., 2008. DOI:10.1007/s11046-007-9048-1

Calich VLG, Pina A, Felonato M, Bernardino S, Costa, Loures FV. 2008. Toll-Like Receptors and Fungal Infections: The role of TLR2, TLR4 and MYD88 in Paracoccidioidomycosis. *FEMS Immunol. Med. Microbiol*. ID: 402875-141733 or ID: FEMSIM-07-11-0297

Pina A, Bernardino S, Calich VLG. 2008. Alveolar macrophages from susceptible mice are more competent than those of resistant mice to control initial *Paracoccidioides brasiliensis* infection. *J. Leuk. Biol.* DOI:10.1189/jlb.1107738.

Vera Lúcia Garcia CALICH

Instituto de Ciências Biomédicas
Universidade de São Paulo (USP)
Avenida Prof. Lineu Prestes, 1730 – Butantã
CEP 05508-900 – São Paulo, SP – Brasil

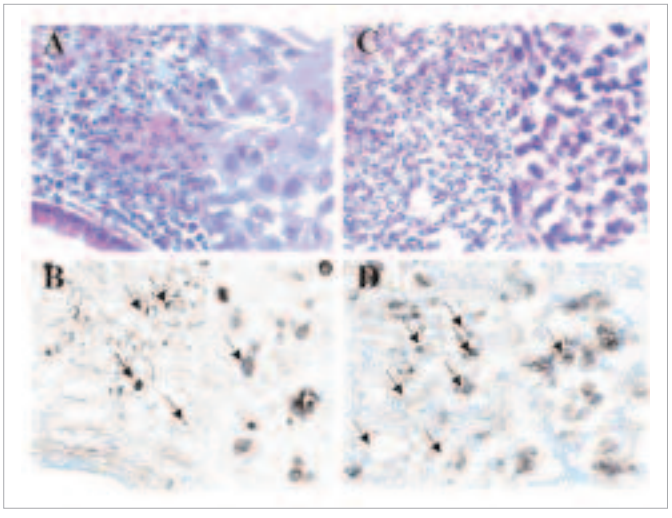
+55-11-3091-7397
vlcalich@icb.usp.br

MODULATION OF INNATE AND ACQUIRED IMMUNE RESPONSE BY LEUKOTRIENES AND PROSTAGLANDINS

Lúcia Helena FACCIOLI

Ribeirão Preto School of Pharmaceutical Sciences / University of São Paulo (USP)

Several cells, in response to various stimuli, produce arachidonic acid (AA) metabolites. AA released can either be metabolized by cyclooxygenase enzymes 1 and 2 (COX1 and COX2) or by one of the lipoxygenases (LO), among them 5-lipoxygenase (5-LO), originating prostaglandins (PGs) and leukotrienes, respectively. PGs induce pain, vasodilatation, increase in vascular permeability, fever, and regulate the production of cytokines and the inflammatory process. On the other hand, some cytokines are able to regulate the production of PGs, and this magnifies the importance of studies involving these mediators and cytokines, which play a role in innate and acquired immune response. Leukotrienes induce leukocyte recruitment, plasma extravasation, mucus secretion, vascular relaxation, vasoconstriction and bronchoconstriction in different inflammatory processes. Moreover, leukotrienes play an important role in modulating the synthesis and release of inflammatory cytokines, immune response, also participating in cellular activation and/or production of antibodies. These lipid mediators also modulate microorganism phagocytosis by immune system cells. We have demonstrated that leukotrienes are involved in the systemic eosinophilia in response to infestation by *Toxocara canis*, but do not modulate expression of some adhesion; recruitment of leukocytes in response to intraperitonial inoculation of *Histoplasma capsulatum* (*Br. J. Pharmacol.*, 1999); and leukocyte recruitment in response to inoculation of venom from the wasp *Polybia paulista*. We have studied the participation of leukotrienes in lung infection by *H. capsulatum* and obtained novel and extremely relevant results. We observed that inhibition of the release of leukotrienes results in death of 100% of animals infected by *H. capsulatum* and this phenomenon seems to be related to the intense inflammatory reaction in the lungs and the accentuated proliferation of the fungus. We have also demonstrated that leukotrienes regulate the release of inflammatory cytokines such as IL-1, IL-6, KC and TNFa, and some chemokines, such as MCP-1, RANTES, MIP-1, MIP-2, IP-10, and MCP-1. Moreover, we have demonstrated that leukotrienes are essential for the release of cytokines in the immune response, such as IL-2, IL-12, and IFNy, as well as nitric oxide. In order to extend the observations on the role of AA metabolites as immunoregulators, we aim to investigate the participation of leukotrienes and/or prostaglandins (i) in the protection induced by an exoantigen from *H. capsulatum*; (ii) in infection by *Strongyloides venezuelensis*; (iii) in the release of cytokines and production of immunoglobulins in toxocariasis; (iv) in infection by *Mycobacterium tuberculosis*; (v) in oral tolerance. In addition, one of our goals is to investigate the use of these metabolites as adjuvants and immunomodulators in the treatment of various infectious diseases. There are no reports so far on experiments where leukotrienes and prostaglandins have been administered *in vivo* with these objectives. In this project we also aim to develop microspheres and nanoparticles containing leukotrienes and/or prostaglandins for *in vivo* administration.

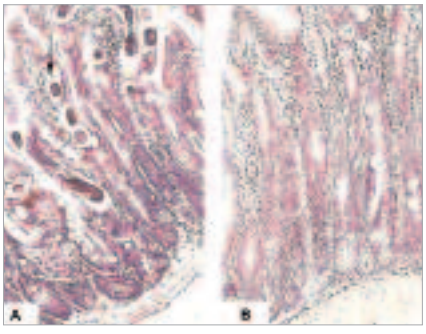


Increased leukocyte recruitment and fungal burden in the lung parenchyma resulting from endogenous leukotriene inhibition using MK886. Representative lung sections from mice infected with H. capsulatum *(Hc) and receiving p.o. water (A) or MK 886 daily. (C) H&E staining for leukocytes; (A and C) and GMS staining (B and D) for yeast cells (black arrow). Magnifications: ?360 (A and B) and ?900 (C and D). (Medeiros et al., 2004).*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Leukotrienes (LTs) comprise a family of lipid products of the 5-lipoxygenase (5-LO) pathway of arachidonic acid metabolism. The two principal bioactive classes of LTs are LTB4 and cysteinyl LTs (cysLTs) which are well known for their participation in inflammatory diseases and in protective immune responses in infection.

Using pharmacological tools (compound Mk886) to inhibit LT synthesis or mice with deficiency of 5-LO-/- enzyme (5-LO-/-), we have demonstrated that these lipid mediators are crucial to effective host defense against *Histoplasma capsulatum*, *Toxocara canis*, *Strongyloides venezuelensis* and *Mycobacterium tuberculosis*. Partial or total absence of LT had an important impact on cell recruitments and in cytokines, nitric oxide (NO) and antibodies productions, resulting in a defective immune response and in an increase of pathogen replication, and worsening the infections.



Histopathology of duodenum from S. venezuelensis*-infected 5-LO-/- (A) or Wild Type (B) mice. The arrows indicate sections of adult worms. The tissues were collected 14 days post infection and stained with H&E. Magnification of panels x100. (Machado et al, 2005)*

Based in ours results we have proposed the use of LTs as adjuvant or immunomodulators to control the immune responses in infectious diseases. We have developed a new LTB4-based pharmacological formulation in PLGA micro spheres (MS-LTB4) as a controlled release system to study the effects of LTs in in vitro and in vivo experimental models. We have demonstrated that MS-LTB4 formulations protects LTB4 from degradation and assure the delivery of the lipid mediator to the site of infection, inducing increased in cell recruitment, phagocytosis, and NO and cytokines productions. Moreover, MS-LTB4 injection protected animals against lethal infection with *H. capsulatum*, suggesting that the use of micro particulate system can be an advantageous tool to *in vivo* delivery of LTs.

With this project we intend to understand better the role of LTs in the immune response during infectious diseases and to develop controlled release formulations containing LTs to be used as therapeutic co-adjuvants for the treatment of infectious diseases.

MAIN PUBLICATIONS

Medeiros AI, Sá-Nunes A, Turato W, Secatto A, Frantz FG, Sorgi C, Serezani CH, Deepe, Jr G. and Faccioli LH. 2008. Leukotrienes are potent adjuvant during fungal infection. *Effects on memory T cells*. Submmited.

Sorgi CA, Medeiros, AI, Secatto A, Fontanari C, Turato, W, Belangér C, Maleau S, Haad SK, Covas D, Bozza P and Faccioli LH. 2008. *Histoplasma capsulatum* cell wall ß-glucan induced lipid body formation and LTB4 generation trough TLR2 and CD18 receptors: a correlation with HIV-1infection. Submmited.

AnibaL F, Rogério AP, Malheiro A, Machado ER, Martins-Filho OA, Andrade MC, Soares EG, Medeiros AI, Faccioli LH. 2007. Impact of MK886 on Eosinophil counts and phenotypic features in toxocariasis. *Scand. J. Immunol*. 344-352.

Nicolete R, Lima KM, Junior JMR, Baruffi MD, Medeiros AI, Bentley MV, Silva CL, Faccioli LH. 2007. *In vitro* and *in vivo* activities of leukotriene B4-loaded biodegradable micro spheres. *Prostaglandins & other Lipid Mediators*. 121-129.

Peres CM, Paula L De, Medeiros AI, Sorgi CA, Soares EG, Carlos D, Peter-Golden M, Silva CL, Faccioli LH. 2007. Inhibition of leukotriene biosynthesis abrogates the host controlo f *Mycobacterium tuberculosis*. *Microbes and Infection*. 483-489.

Peres CM, Aronoff DM, Serezani CH, Flamand N, Faccioli LH, Peters-Golden, M. 2007. Specific leukotriene receptors couple to distinct G Proteins to effect stimulation of alveolar macrophage host defense functions. *J. Immunol*., 5454-5461.

Machado ER, Ueta MT, Lourenço EV, Anibal FF, Sorgi CA, Soares EG, Roque-Barreira MC, Medeiros AI, Faccioli LH. 2005. Leukotrienes play a role in the control of parasite burden in murine Strongyloidiasis. *J. Immunol*. 3892-3899.

Sá-Nunes A, Medeiros AI, Faccioli LH. 2004. Interleukin-5 mediates peritoneal eosinophilia induced by the F1 cell wall fraction of *Histoplasma capsulatum*. *Braz. J. Med. Biol Rer*. 343-346.

Medeiros AI, Sá-Nunes A, Soares EG, Peres CM, Silva CL, Faccioli LH. 2004. Blockade of endogenous leukotrienes exacerbates pulmonary histoplasmosis. *Infection Immunity*. 1637-1644.

Lúcia Helena FACCIOLI

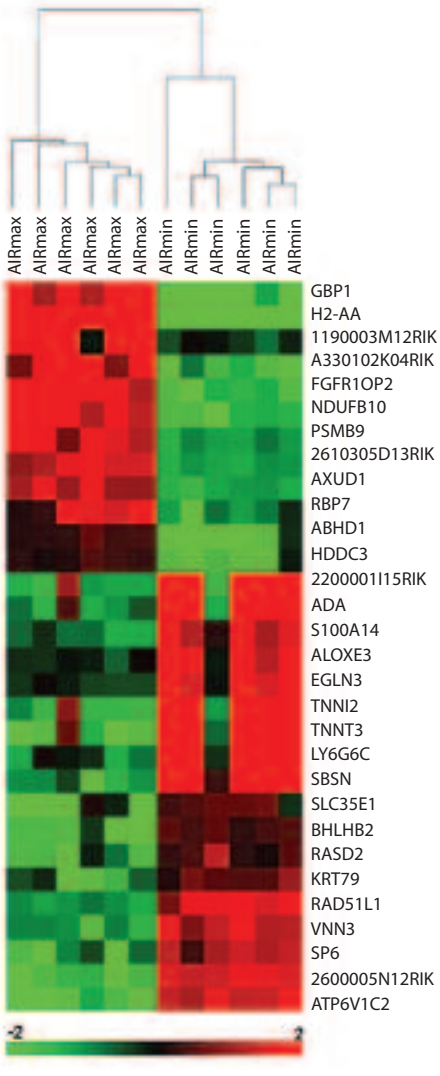
Faculdade de Ciências Farmacêuticas de Ribeirão Preto
Universidade de São Paulo (USP)
Avenida do Café s/n – Monte Alegre
14040-903 – Ribeirão Preto, SP – Brasil

+55-16-3602-4303
faccioli@fcfrp.usp.br

IDENTIFICATION OF GENETIC FACTORS AFFECTING RESISTANCE AND SUSCEPTIBILITY TO CHEMICAL CARCINOGENESIS AND THE DEGREE OF ACUTE INFLAMMATORY RESPONSE, BY USING A MODEL OF GENETICALLY SELECTED MOUSE LINES

Olga Célia Martinez IBANEZ

Butantan Institute



Genes differentially expressed in the normal lung tissue in AIRmax and AIRmin mice

Two non isogenic mouse lines phenotypically selected according to the maximal (AIRmax) or minimum (AIRmin) acute inflammatory response (AIR) show differential susceptibility to chemical carcinogenesis in the lungs and skin, AIRmax being resistant and AIRmin susceptible. As a consequence of the bi-directional selective process, the alleles of the genes relevant to the "maximal" and "minimum" inflammatory response phenotypes, specifically segregated in AIRmax and AIRmin lines, respectively, leading to homozygosity in AIR modifier *loci* but maintaining the background genetic heterogeneity in each line. The analysis of the heritability of the character during the bi-directional selective process, revealed the involvement of about 11 Quantitative Trait Loci (QTL) with additive effect, accounting for the phenotypic divergence between AIRmax and AIRmin mice.

Our project aims to evaluate whether functional polymorphisms in genes, which could explain the variations in the inflammatory response in the two selected mouse lines, harbor an altered risk to the development of neoplasic diseases. In order to identify these genes, complementary strategies will be applied:

1) the search of polymorphism in candidate genes indicated from precise phenotypes relevant to inflammatory response regulation and to chemical carcinogenesis predisposition which differentiate the two lines.

2) genome-wide screening using polymorphic genetic markers between the two lines for the identification of the fine chromosomal location of candidate genes.

3) comparative transcriptome and proteome analysis of the inflammatory exudates, bone marrow and target tissues preparations from AIRmax and AIRmin mice, before and after the stimulus with inflammatory or carcinogenic agents.

The expression profiling data will be then combined to the marker-based QTL mapping. This analysis might unravel gene-gene interactions that affect the inflammatory phenotypes and cancer resistance or susceptibility of the two lines. The identification of functional polymorphisms in genes relevant to the variations in inflammatory response could be useful for the assessment of genetic cancer risk factors and eventually to indicate their human counterparts.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The biological significance of this model was evidenced by the huge differences found between the two mouse lines in bone marrow granulopoietic activity and in the natural resistance to various infections and autoimmune diseases. Furthermore, AIRmax mice are considerably more resistant than AIRmin to skin and lung tumorigenesis, independent of the chemical nature or the route of administration of the carcinogen, indicating a broad effect of germ line genes segregated in these mice in innate resistance to tumorigenesis. In fact, "high" AIR co-segregated with resistance and "low" AIR with susceptibility to skin and lung tumorigenesis in two independent co-segregation assays carried out in F2 (AIRmax x AIRmin) intercross populations, demonstrating that at least part of the genes which determine the degree of acute inflammation are, or are closely, linked to cancer modifier genes.

In this context we could demonstrate the segregation of resistance or susceptibility related alleles in a 452-kb region, containing 5 genes in linkage located at the distal region of chromosome 6 which regulate lung tumorigenesis (*Pas1* locus). We identified also polymorphism at aryl hydrocarbon receptor coding gene in chromosome 12 which is related to variations in the receptor affinity to polycyclic aromatic hydrocarbon carcinogens resulting in resistance (AIRmax) and in susceptibility (AIRmin) to skin and lung tumorigenesis by these compounds. Linkage analysis showed also, originally, the functional involvement of these gene loci in inflammatory response regulation.

Due to this grant we established collaboration with Dr. Tommaso Dragani at the Polygenic Inheritance Unit at Istituto dei Tumori in Milan, Italy, for the introduction in our laboratory at Institute Butantan of techniques for large scale genetic analysis. Micro arrays are being used for gene expression analysis in target organs and the results are validated by real-time PCR and confronted to the proteome profiles obtained in the same organs. Results in the bone marrow, in the lungs and skin of normal or carcinogen treated AIRmax and AIRmin mice revealed line-specific patterns of gene expression associated to inflammatory response.

Micro arrays containing thousands of oligonucleotides for the analysis of single nucleotide polymorphisms (SNP) are also being used for whole genomic screening of the two mouse lines in pedigree studies using parental (AIRmax and AIRmin), F1 hybrids and F2 intercross populations.

MAIN PUBLICATIONS

Ribeiro OG, Maria DA, Adriouch S, Pechberty S, Cabrera WH, Morisset J, Ibanez OM and Seman M. 2003. Convergent alteration of granulopoiesis, chemotactic activity, and neutrophil apoptosis during mouse selection for high acute inflammatory response. *Journal of Leukocyte Biology*. **74(4)**:497-506, 2003.

Maria DA, Manenti G, Galbiati F, et al. 2003. Pulmonary adenoma susceptibility (*Pas1*) locus affects inflammatory response. *Oncogene.* **22**:426-432.

de Souza CM, Morel L, Cabrera WH, et al. 2004. Quantitative trait loci in Chromosomes 3, 8, and 9 regulate antibody production against *Salmonella flagellar* antigens in the mouse. *Mammalian Genome.* **8**:630-636.

Zaffaroni D, Spinola M, Galvan A, Falvella FS, et al. 2005. *Met* proto-oncogene juxtamembrane rare variations in mouse and humans: differential effects of Arg and Cys alleles on mouse lung tumorigenesis. *Oncogene.* **24**:1084-1090.

Ribeiro OG, Cabrera WH, Maria DA, et al. 2005. Genetic selection for high acute inflammatory response confers resistance to lung carcinogenesis in the mouse. *Experimental Lung Research*. **31(1)**: 105-116.

Santos Junior RR, Sartori A, Ribeiro OG, et al. 2005. Immunomodulation and protection induced by DNA-hsp65 vaccination in an animal model of arthritis. *Human Gene Therapy*. USA. **16**: 1338-1345.

LaPrete AC, Maria DA, Rodrigues DG, et al. 2006. Evaluation in melanoma bearing mice of an etoposide derivative associated to a cholesterol-rich nanoemulsion. *Journal of Pharmacy and Pharmacology*. **58(6)**: 801-808.

Di Pace RF, Massa S, Ribeiro OG, Cabrera WHK, et al. 2006. Inverse genetic predisposition to colon vs. lung carcinogenesis in mouse lines selected based on acute inflammatory responsiveness. *Carcinogenesis*. **27 (8)**:1517-1525.

Peters LC, Jensen JR, Borrego A, Cabrera WHK, et al. 2007. *Slc*11A1 (formerly *Nramp*1) gene modulates both acute inflammatory reactions and pristane-induced arthritis in mice. *Genes and Immunity.* **8**: 51-56.

10- De Franco M, Carneiro OS, Peters LC, et al. 2007. *Slc*11A1 (*Nramp*1) alleles interact with acute inflammation loci to modulate wound-healing traits in mice. *Mammalian Genome*. **18(4)**:263-9.

Olga Célia Martinez IBANEZ

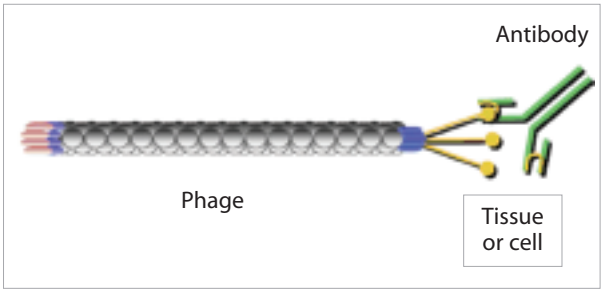
Instituto Butantan
Avenida Vital Brasil, 1500 – Butantã
05503-900 – São Paulo, SP – Brasil

+55-11-3726-7222 r. 2121
olgaibanez@butantan.gov.br

PHAGE DISPLAY AND IMMUNE SYSTEM: IDENTIFICATION OF MOLECULAR TARGETS WITH DIAGNOSTIC AND THERAPEUTIC PROPERTIES IN IMMUNOLOGICAL DISORDERS

Jorge Elias KALIL Filho

Heart Institute (InCor)

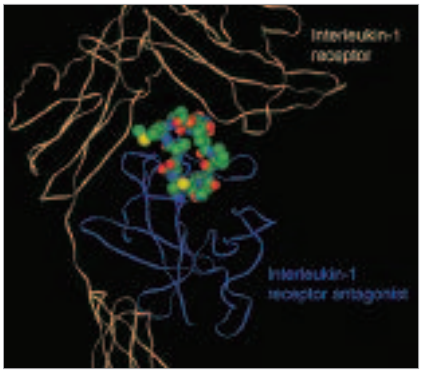


The different panning strategies used in the project: phage libraries used to select for antibodies or molecules on cell or tissue surface

Peptide phage display is a powerful tool that utilizes bacteriophages to screen cells, tissues or organs in search of receptor-pair ligands. Phage display particles are like biological nanoprobes, capable of interacting with the target though small peptides, genetically engineered to be expressed on the virus outer coat. Since the peptide sequence information is coded by bacteriophage genome, a single virus particle bound to a cell or tissue sample can be recovered by bacterial infection, amplified and the peptide identified. Few techniques allow studying molecular interactions of single molecules or cell without previous knowledge of the target. The aim of this project is to establish a web of knowledge and think tank in phage display technology in Brazil. The technique will be employed at full capacity, in different approaches but the main theme will be the immune system. Specifically: identification of epitopes in humoral response (IgG) to Papilomavirus and acute renal vascular rejection (Component A); identification of auto immune CD4+ T cell epitopes involved in the rheumatic fever disease (Component B); inflammation and endothelial cells in the atherosclerotic disease (Component C); B1 lymphocytes and tumor growth (Component O); thymus and regulatory cells development (Component E); study of the structural behavior of selected peptides and the dynamic of interaction with their respective receptors by nuclear magnetic ressonance. Below are the experimental details of each component of the project.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Immune recognition of self and non-self relies on the molecular biodiversity among other factors. Proteins and peptides are important components and orchestrators of this process, helping and guiding the cellular and humoral repertoire of our immunological system to distinguish between self and pathogenic. However, several diseases originate due to an imbalance in this intricate network of immune cells, antibodies and cytokines. Combinatorial technologies, such as phage display, are formidable tools to probe and investigate such disorders. Due to its high throughput and unbiased nature, peptide phage display libraries allow researchers to screen among millions of peptide permutations in order to identify antigens and epitopes involved in health and disease. These results can then be brought to the clinic in the form of new vaccines, antibody therapies or helping in the design of a new generation of safer and more efficient drugs. The aim of our project is to use phage display technology to study disorders of the immune system associated with specific pathogens (Papiloma virus and *S. pyogenes*), allograph rejection (kidney), and the endothelial dysfunction observed in atherosclerotic vessels. We also use phage display to study CD4CD25 regulatory T cells, important controllers of the immune system and whose function is affected in several diseases. Our goal is to understand the molecular mechanisms for the progression of these disorders, hoping that our results will, in time, translate into new and better therapeutic agents for the treatment of such disorders. In order to achieve our goal, the following studies are currently being conducted:



Peptides identified by phage display suggest a prominent role for interleukin-1 and family members in the pathogenesis of rheumatic fever

- Identification of antibody epitopes by phage display: Peptide sequences recognized by antibodies of the serum of HPV infected patients;
- Identification of antigens involved in acute vascular kidney rejection;
- Identification of T cell epitopes by phage display: Rheumatic fever as model;
- Identification of peptide ligands of activated endothelium: Atherosclerotic disease as a model;
- Identification of surface markers of B1 lymphocytes and melanoma cells: Immunomodulation of neoplasia by B1 lymphocytes, and
- Identification of markers of thymus and CD4+/CD25+regulatory cells in human thymus: Search for relevant molecules in maturation and activity of CD4+/CD25+regulatory cells.

MAIN PUBLICATIONS

All experiments have been concluded, with resulting manuscripts due for publication this year.

Jorge Elias KALIL Filho

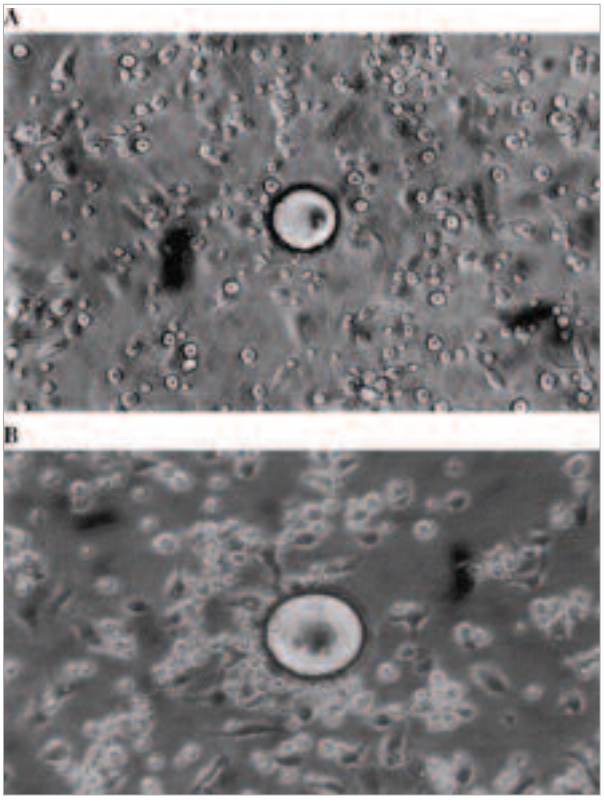
Fundação Zerbini
Faculdade de Medicina / Universidade de São Paulo
Instituto do Coração – Laboratório de Imunologia
Avenida Dr. Enéas de Carvalho Aguiar, 44
Bloco II 9º – Cerqueira Cesar
CEP 05403-001 – São Paulo, SP – Brasil

+55-11-3069-5900
jkalil@usp.br

B-1 CELLS AND THEIR PARTICIPATION IN THE EXPERIMENTAL MODELS OF MURINE AND *Paracoccidioides brasiliensis* INFECTION

José Daniel LOPES

Microbiology, Immunology and Parasitology Departament / Federal University of São Paulo (Unifesp)



Granuloma assays with purified B-1 cells or total adherent cells from Xid mice. gp43-coated polyacrylamide beads incubated with 106 cells and with 50 ìg/ml soluble gp43. The granulomatous reactions were observed on day 7 of culture. (A) Granuloma-like structures were not formed when isolated B-1 cells were used. (B) Total adherent peritoneal cells from Xid *mice were cultured under the same conditions and negative results were also observed. Results are representative of three independent experiments*

After more than 20 years since the discovery of B-1 cells, efforts to characterize their origin and function have led to little understanding about their role in phenomena such as inflammation.

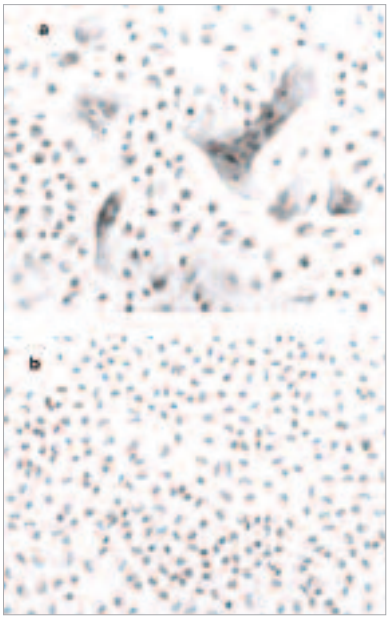
B-1 cells constitute a minor fraction of the B-cell population in spleen and lymph nodes of mice, but are the main B-cell population in their peritoneal and pleural cavities. They express high surface IgM, low B220 and IgD, but not CD23. With features of B lymphocytes, they also express markers of monocyte-derived macrophages such as Mac-1. A subset designed B-1a express CD5. Both antigens are lost when B-1 cells leave the peritoneal cavity. These characteristics poise this lineage as a promiscuous one.

B-1 cells spontaneously proliferate in cultures of normal adherent mouse peritoneal cells. Support for this conclusion came when cultivated peritoneal cells from Xid mice showed no B-1 cell proliferation. B-1 cells migrate to a non-specific inflammatory site and differentiate into a macrophage-like cell. Nevertheless, the role these cells play on the inflammatory response and on parasite infection is not yet established. However, Xid mice are more resistant to *T. cruzi*, *P. brasiliensis* and filarial infections. Thus, B-1 cells could down-regulate the efficacy of effector cells to eliminate parasites in the inflammatory milieu. Also, B-1 cells produce and utilize IL-10, a negative regulator of cell mediated immunity, as an autocrine growth factor.
We showed that B-1 cells influence macrophages effector functions via IL-10 secretion.

We intend to demonstrate that B-1 cells migrate to inflammatory sites, transforming into a novel type of phagocyte. Characterization of their precursors and their mechanisms of survival in culture will also be addressed. Results will certainly bring new insights on the role these cells could play in inflammatory, degenerative and neoplasic pathologies.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Investigation of the origin, properties and fate of B-1 cells is promising in two guises. First, these cells are known to exist in mammalians, mainly in the mice, for a little longer than 20 years. Second, properties of these cells described in the literature point to a still uncovered yet relevant role they may play in physiology and general pathology. For the last 10 years our laboratory has made efforts to build up a comprehensive understanding of the part they play in physiology, basic phenomena as wound healing and graft rejection, models of infection and tumor growth control. Investigation of these themes was possible due to the discovery in our laboratory that B-1 cells grow in cultures of adherent peritoneal cells, and that they are radio-sensitive and migrate from the peritoneal cavity to distant inflammatory foci. Among the most relevant contributions from our group is the unequivocal demonstration that B-1 cells differentiate into a mononuclear phagocyte similar to macrophages. This contribution to the physiology of B-1 cells imposes that nature and function of mononuclear cells, mainly in chronic inflammation, must be revisited. In addition to these observations, the laboratory has demonstrated that B-1 cells are endowed with immunological memory and tolerogenic property when tested in a model of hypersensitivity, and finally, an unexpected effect of B-1 cells was observed when investigated in a model of murine melanoma. B-1 cells have the property of enhancing melanoma transformation, growth and spreading by mechanisms now being uncovered. Attachment of B-1 to melanoma cells parallels ERK activation as demonstrated *in vitro* and *in vivo*. We expect the investigation of B-1 cells in apparently dissimilar models will result in a comprehensive understanding of the origin, properties and fate of these cells.



(a, b) Giant cells are formed on the surface of glass cover slips implanted for 4 days into the subcutaneous tissue of CBA/J mice (a) and are almost absent on glass cover slips implanted, for the same time, in CBA/N *(Xid) mice (b). Mononuclear cell migration and adherence to the glass surface in CBA/N mice is not impaired. Haematoxilin-eosin x400*

MAIN PUBLICATIONS

Pérez EC, Júnior JM, Aliperti F, Freymuller E, Mariano M, Lopes JD. 2008. B-1 lymphocytes increase metastatic behavior of melanoma cells through the extracellular signal regulated kinase pathway. *Câncer Science*. 2008. **99(5)**:920-928.

Mendes PFP, Xander P, Brito RRN, Mortara RA, Jasiulionis MG, Lopes JD. 2008. A cell surface 230 KDa protein from murine melanoma involved with tumor malignancy. *Cancer Letters.* **262**:276-285.

De Lorenzo BHP, Brito RRN, Godoy LC, Lopes JD, Mariano M. 2007. Tolerogenic property of B-1b cells in a model of allergic reaction. *Immunology Letters*. **114**:110-118.

e Brito RRN, De Lorenzo BHP, Xander P, Godoy LC, Lopes JD, da Silva NP, Sampaio SC, Mariano M. 2007. Role of distinct immune components in the radiation-induced abrogation of systemic lupus erythematosus development in mice. *Lupus.* **16**:947-954.

Xander P, Vigna AF, Feitosa LS, Pugliese L, Bailão AM, Soares CMA, Mortara RA, Mariano M, Lopes JD. 2007. A suface 75-KDa protein with acid phosphatase activity recognized by monoclonal antibodies that inhibit *Paracoccidioides brasiliensis* growth. *Microbes and Infection* **9**:1484-1492.

Vigna AF, Almeida SR, Xander P, Freymuller E, Mariano M, Lopes JD. 2006. Granuloma formation *in vitro* requires B-1 cells and is modulated by *Paracoccidioides brasiliensis* gp43 antigen. *Microbes and Infection.* **8**:589-597.

Marques-da-Silva SH, Colombo AL, Blotta MHSL, Queiroz-Telles F, et al. 2006. Diagnosis of Paracoccidioidomycosis by detection of antigen and antibody in bronchoalveolar lavage fluids. *Clinical and Vaccine Immunology.* 1363-1366.

Bogsan CSB, Brito RRN, Palos MC, Mortara RA, et al. 2005. B-1 cells are pivotal for *in vivo* inflamatory giant cell formation. *International Journal of Experimental Pathology*. **86(4)**:257-265.

Popi AF, Lopes JD, Mariano M. 2004. Interleukin-10 secreted by B-1 cells modulate the phagocytic activity of murine macropages *in vitro*. *Immunology*. **113**:348-354.

José Daniel LOPES

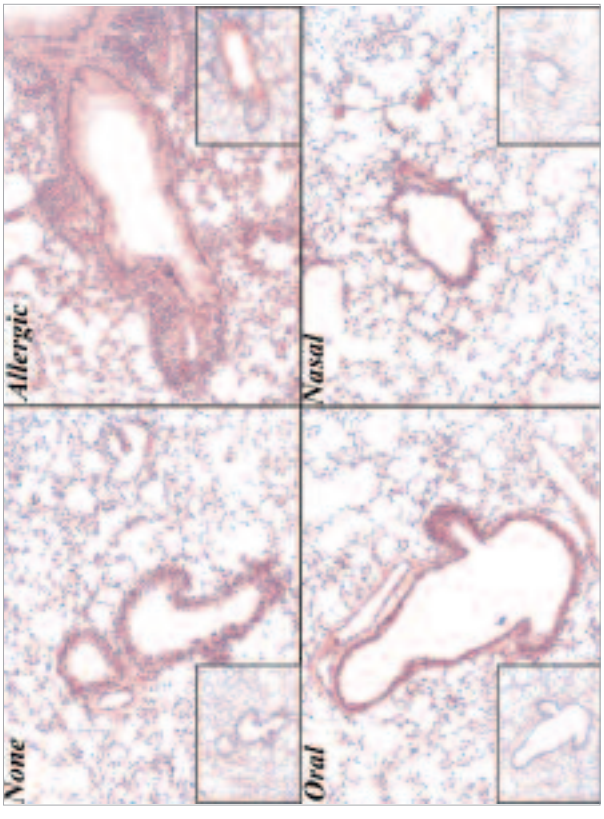
Universidade Federal de São Paulo (Unifesp)
Depto de Microbiologia, Imunologia e Parasitologia
Rua Botucatu, 862, 6º andar, Vila Clementino
CEP 04023-901 – São Paulo, SP – Brasil

+55-11-5549-6073
jdlopes@ecb.epm.br

ACTIVATION/DEACTIVATION OF MACROPHAGES AND CD4+ T LYMPHOCYTES IN EXPERIMENTAL ASTHMA

Momtchilo RUSSO

Institute of Biomedical Sciences / University of São Paulo (USP)



Modulation of experimental asthma by oral or nasal olerance. The figure shows lungs of healthy, allergic and animals treated with allergens by oral and nasal route

Over the last 2 to 3 decades, the prevalence and severity of asthma and allergic disease has increased extensively in urban environments. The "hygiene hypothesis" was proposed in order to explain this increased incidence. This hypothesis is based on epidemiological evidence showing an inverse correlation between infections and allergic or autoimmune diseases. Recently, it was proposed that infections generate regulatory T cells that inhibit allergic (Th2) or autoimmune (Th1) diseases. Accordingly, the control of infectious diseases in urban environments decreased Treg cells activities that lead to a deregulated immune system. Various Treg cells has been characterized, natural Treg (CD4+ CD25+ CD45RB low) which inhibit aureactive T cells and adaptive Treg, that inhibit Th1- or Th2-mediated immune reactions. The adaptive Treg are heterogeneous cell population, but natural Treg cells are CD4+ CD25+ and the majority expresses the transcription factor Foxp3. One objective of the project is to investigate whether Treg induced by oral or nasal tolerance, or by recombinant BCG infection, or LPS would suppress the development of asthma. Another possibility for the suppression of asthma by infections is the emergence of activated macrophages. Recently, we have shown that i.v. administration of LPS suppresses established asthma. The suppressive activity was dependent on TLR4 and NOS2 expression and NO production. It is possible that other metabolites produced by activated macrophages such as those derived from indolamine, 2,3 dioxigenase or hemeoxigenase 1 pathways may also suppress asthma. For this purpose we will test some classical TLRs agonists (PIC, LPS or rBCG), or a synthetic TLR4-agonist on the development of asthma with focus on these metabolic pathway.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Usually, the immune system protects against a variety of noxious agents. However, in certain circumstances it can cause disease. Some immunological disorders are due to a polarization of T helper cells that can be characterized as Th1 or Th2 cells. Th1 cells are involved in autoimmune diseases such as Crohn's disease and arthritis, while Th2 cells mediate allergic processes such as atrophy and asthma. Experimentally, it is possible to reproduce these diseases using different types of adjuvants. Our focus is to understand the modulation of Th2 polarized allergic response, using essentially a murine model of asthma, either by interfering with the adjuvant activity or by manipulating the immune system through immunological tolerance or microbial products.

We have shown that the asthma-like responses could be down modulated by interfering in the adjuvant activity of Alum, the most commonly used adjuvant in human vaccination and a prototypic Th2 adjuvant (Bortolatto, 2008). In another work, we showed that the acute phase protein, serum amyloid A could be a TLR4 agonist, or a Th1 adjuvant (Sandri, 2008). Taken together, our work opens the possibility of using a combination of adjuvants to induce protective immune responses without polarization. We also showed that natural products such as the plant *Lafoensia pacari* have anti-inflammatory activity in our asthma model (Rogerio, 2008). Another line of investigation deals with immunological tolerance that is achieved by the administration of allergen via mucosal surfaces (Keller, 2006 and Mucida 2005). We also determined the repercussions of allergy on brain activity. We showed that the brain and behavior are modified by allergic responses (Costa-Pinto, 2007). Finally, products of activated macrophages might be key elements in suppressing allergic responses (Keller, 2005). The impact of our work was acknowledged in a recent publication in *Nature Review Immunology* (March 2008) dealing with allergy.

MAIN PUBLICATIONS

Bortolatto J, Borducchi E, Rodriguez D, Keller AC, et al. Toll-like receptor 4 agonists adsorbed to aluminum hydroxide adjuvant prevent ovalbumin-specific allergic airway disease. *Clinical & Experimental Allergy*. Em revisão final, Março 2008.

Sandri S, Rodriguez D, Gomes E, Monteiro HP, Russo M, Campa A. 2008. Is serum amyloid A an endogenous TLR4 agonist? *Journal of Leukocite Biology*. 83. [Epub ahead of print].

Rogerio AP, Fontanari C, Borducchi E, Keller AC, et al. 2008. Anti-inflammatory effects of *Lafoensia pacari* and ellagic acid in a murine model of asthma. *European Journal Pharmacology*. **580**:262-70.

Ferreira KS, Bastos KR, Russo M, Almeida SR. 2007. Interaction between *Paracoccidioides brasiliensis* and pulmonary dendritic cells Induces Interleukin-10 production and Toll-like receptor-2 expression: possible mechanisms of susceptibility. *The Journal of Infectious Diseases*. **196**:1108-15.

Rosa DS, Bastos KR, Bargieri DY, Tzelepis F, et al. 2007. Role of interferon-γ during CpG oligodeoxynucleotide-adjuvanted immunization with recombinant proteins. *Vaccine*. **25**:6007-17.

Costa-Pinto FA, Basso AS, Russo M. 2007. Role of mast cell degranulation in the neural correlates of the immediate allergic reaction in a murine model of asthma. *Brain, Behavior, and Immunity*. **21**:783-90.

Calderón L, Facenda E, Machado L, Uyema K, et al. 2006. Modulation of the specific allergic response by mite allergens encapsulated into lipossomes. *Vaccine*. **24. Suppl 2**:S2-38-9.

Keller AC, Mucida D, Gomes E, Faquim-Mauro E, Faria AMC, Rodriguez D, Russo M. 2006. Hierarchial suppression of asthma-like responses by mucosal tolerance. *J. Allergy Clin. Immunol.* **117**:283-90.

Mucida D, Kutchukhidze N, Erazo A, Russo M, Lafaille J, Curotto de Lafaille AM. 2005. Oral tolerance in the absence of naturally occurring Tregs. *J. Clin. Investigation*. **115**:1923-33.

Keller AC, Rodriguez D, Russo M. 2005. Nitric Oxide paradox in asthma. *Mem. Inst. Oswaldo Cruz*. **100 (Suppl I)**: 19-23.

Momtchilo RUSSO

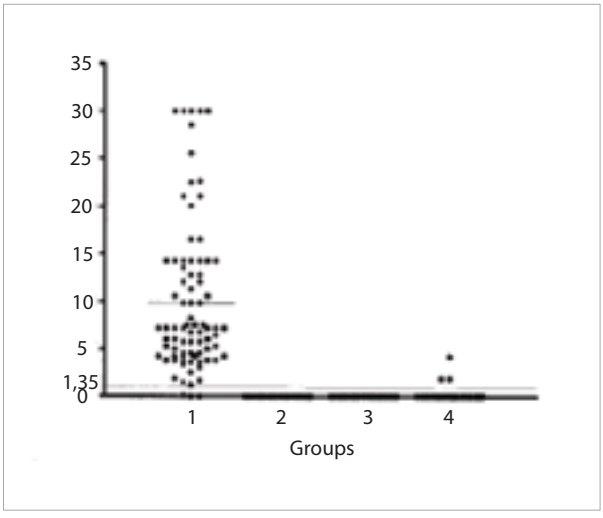
Instituto de Ciências Biomédicas
Universidade de São Paulo (USP)
Departamento de Imunologia
Avenida Prof. Lineu Prestes, 1730 – C. Universitaria
05508-900 – São Paulo, SP – Brasil

+55-11-3091-7377
momrusso@usp.br

EMERGENT FUNGAL PATHOGENS: EPIDEMIOLOGY AND DIAGNOSIS

Zoilo Pires de CAMARGO

Microbiology, Immunology and Parasitology Departament / Federal University of São Paulo (Unifesp)

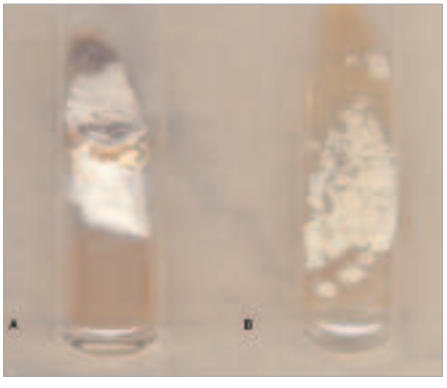


Detection of circulating antigen in sera from patients with PCM or with other mycoses and in normal human sera by inh-ELISA. Groups studied 1, PCM (n=81); 2, cryptococcosis (n=20); 3 histoplasmosis (n=33); 4, normal human sera (n=93). Bars represent the mean antigen concentration for each group. The long, fine line represents the cutoff point (1.35 µg/ml)

Fungal infections have been one of the main factors of morbidity and death in patients with various diseases. During the last years great progresses were obtained in the treatment of immunossupressed patients and the proportion of patients with complete cure and survival have been increased. Many oportunistic fungal infections are caused mainly by *Candida albicans* and *Aspergillus fumigatus*. However, an increased number of pathogenic fungi such as *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *Trichosporon spp.*, *Sporothrix schenckii*, *Histoplasma capsulatum*, *Coccidioides immitis* and *Paracoccidioides brasiliensis* have been observed. In order to reach knowledg that may aid the patients, epidemiological, antigenic aspects and improvement of the mycological diagnosis will be studied.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Paracoccidioidomycosis (PCM) is an important systemic fungal disease, particularly among individuals living and working in rural areas of endemic in Latin America, who, without antifungal therapy, may develop fatal acute or chronic infection. For such patients, the detection of antibody responses by immunodiffusion is of limited value due to false-negative results. In contrast, the detection of *Paracoccidioides brasiliensis* gp43 circulating antigen may represent a more practical approach to the rapid diagnosis of the disease. Accordingly, an inhibition enzyme-linked immunosorbent assay (inh-ELISA) was developed for the detection of a 43-kDa *P. brasiliensis* – specific epitope incorporating a species-specific murine monoclonal antibody. With sera from patients with acute and chronic forms of the disease (n = 81), the overall sensitivity of the test was found to be 95.1%, while specificity was found to be 97.5% compared to that with normal human sera from blood donors (n = 93) and sera from patients with other chronic fungal infections (histoplasmosis [n = 33] and cryptococcosis [n = 20]). The inh-ELISA detected circulating antigen in 100% of patients with the acute form of PCM and in 95.31 and 100% of patients with the chronic multifocal and unifocal forms of PCM according to the patient's clinical presentation. Cerebrospinal fluid from 14 patients with neuroparacoccidioidomycosis and 13 samples of bronchoalveolar rinsed fluid from patients with pulmonary unifocal PCM were also tested for gp43 detection, with the test showing 100% sensitivity and specificity. This novel, highly specific inh-ELISA represents a significant addition to the existing tests for the diagnosis of PCM.



P. brasiliensis *at room temperature (A) and 37°C (B)*

MAIN PUBLICATIONS

Marque-da-Silva SH, Colombo AL, Blotta MHSL, et al. 2006. Diagnosis of paracoccidioidomycosis by detection of antigen and antibody in bronchoalveolar lavage fluids. *Clin and Vaccine Immunol.* **13**:1363-1366.

Albuquerque C, Marques-da-Silva SHM, Camargo ZP. 2005. Improvement of the specificity of an enzyme-linked immunosorbent assay for diagnosis of paracoccidioidomycosis. *J. Clin Microbiol.* **43**:1944-1946.

Marques-da-Silva SHM, Colombo AL, Blotta MHSL, et al. 2005. Neuroparacoccidioidomycosis diagnosis by circulating antigen and antibody detection in cerebrospinal fluid. *J. Clin. Microbiol.* **43**:4680-4683.

Berzaghi R, Marques-da-Silva SH, Camargo ZP. 2005. Variable gp43 secretion by *Paracoccidioides brasiliensis* clones obtained by two different culture methods. *J. Clin. Microbiol.* **43**:491-493.

Berzaghi R, Amaral CC, Marques-da-Silva SH, Camargo ZP. 2005. Simplified method to produce *Paracoccidioides brasiliensis* exoantigens for immunodiffusion tests. *Med. Mycol.* **41**: 539-542.

Marque-da-Silva SH, Grosso DM, Lopes JD, et al. 2004. Detection of *Paracoccidioides brasiliensis* gp70 circulating antigen and follow up of patients under antimycotic therapy. *J. Clin. Microbiol.* **42**:4480-4486.

Marques-da-Silva SH, Queiroz-Telles F, Colombo AL, Blotta MHSL, Lopes JD, Camargo ZP. 2004. Monitoring gp43 antigenemia in paracoccidioidomycosis patients during therapy. *J Clin Microbiol.* **42**:2419-2424.

Feitosa LS, Cisalpino PS, Santos MRM, Barros TF, et al. 2003. Chromossomal Polymorphism, syntenic relationships, and ploidy in the pathogenic fungus *Paracoccidioides brasiliensis*. *Fungal Gen Biol.* **39**:60-69.

Marques-da-Silva SH, Colombo AL, Blotta MHSL, et al. 2003. Detection of circulating gp43 antigen in serum, cerebrospinal fluid, and bronchoalveolar lavage fluid of patients with paracoccidioidomycosis. *J Clin Microbiol.* **41**: 3675-3680.

Fernandes GF, Deps P, Yamashita JT, Camargo ZP. 2004. IgM and IgG antibody response to *Paracoccidioides brasiliensis* in naturally infected wild armadillos (*Dasypus novemcinctus*). *Med Mycol.* **42**:363-368.

Zoilo Pires de CAMARGO

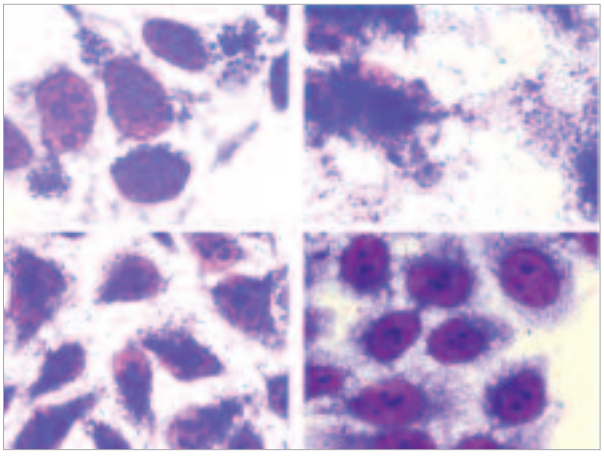
Universidade Federal de São Paulo (Unifesp)
Depto de Microbiologia Imunologia e Parasitologia
Rua Botucatu, 862 – Vila Clementino
04023-062 – São Paulo, SP – Brasil

+55-11-5576-4523 ext. 26
zoilo@ecb.epm.br

ATYPICAL ENTEROPATHOGENIC *Escherichia coli* (ATYPICAL EPEC)

Waldir Pereira ELIAS Júnior

Butantan Institute

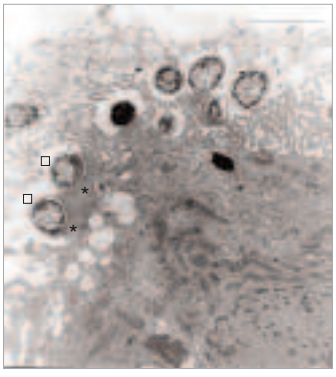


Adherence patterns to HEp-2 cells displayed by aEPEC strains after 6 hours of bacteria-HEp-2 cells interaction. A, localized-like adherence (LA-L); B, aggregative adherence (AA); C, diffuse adherence (DA); D, non-adherence (NA)

The term atypical EPEC is used to define the EPEC strains that does not have the EAF plasmid. This EPEC category has been found in association with diarrhea in several countries, including Brazil. At present they are the most common bacterial agent in child endemic diarrhea in Brazil. Atypical EPEC strains may belong to serotypes of the classical EPEC. The serogroups (classical serotypes) or to serotypes in other the serogroups (non classical serotypes). The classical atypical EPEC serotypes have been identified in several studies but the non-classical ones are less known. The classical serotypes are genetically related to *E. coli* O157:H7 and like this serotype have been isolated from bovines and other animal species. The most common virulence factors identified so far in atypical EPEC strains are the toxins EAST and Hly but recent studies suggest that there are many others factors. The objectives of this project may be divided in two groups: one group is aimed at determining the virulence mechanisms of these organisms and includes studies on their toxins, adhesins, genetic of these virulence factors, interaction mechanisms with the host cell, cell response and proteomics. The other group of objectives includes the characterization of the serotypes and clonal groups of atypical EPEC, genetic relationships of the human and animal strains an development of animal models.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The present study is focused on distinct aspects of atypical enteropathogenic *Escherichia coli* (aEPEC). Initially, the prevalence of aEPEC was determined in a study of the etiology of infantile acute diarrhea in Salvador (BA), where aEPEC was the second most prevalent bacterial pathogen, indicating the emergence of this pathotype in our country. Characterization of 90 aEPEC strains showed that they belonged to 62 different serotypes and presented 14 different intimin subtypes. Adherence assays showed that 42% of them did not adhere to HEp-2 cells and the remaining strains presented localized-like, diffuse, aggregative, localized or undetermined adherence patterns, which were also maintained in intestinal cell lines in culture. The analysis of the attaching-effacing (A/E) caused by aEPEC detected a delay in the bacterial adherence and the A/E formation that correlated to the late expression of intimin, Tir and EspA, caused by the lack of the perABC regulator. Genetic studies showed that genes encoding different virulence factors of other diarrheagenic *E. coli* pathotypes were present in different frequencies. Analysis of genetic relations among strains of aEPEC of human and animal (rabbit, monkey, bovine, ovine, cat and dog) origins suggested that these animals are aEPEC reservoirs. Also, an antiphagocytic effect induced by aEPEC was observed in macrophages J774A1. The enterohemolytic activity of aEPEC presented new features regarding its expression in different culture media and fibronectin binding capacity. Antisera against different adhesins and toxins were produced and employed in immunological assays aiming the differentiation of aEPEC from typical EPEC and EHEC. Other aspects of this project, such as proteomic analysis, biofilm formation, plasmids and adhesins characterization, and development of a murine model of infection are still in progress.



*Transmission electron microscopy showing the attaching and effacing (A/E) lesion of aEPEC (strain 558 /serotype O111:H40) on polarized Caco-2 cells after 6 hours of interaction. □ indicate bacteria; * indicate pedestal (A/E lesion)*

MAIN PUBLICATIONS

Garmendia J, Ren Z, Tennant S, Vieira MAM, Chong Y, Whale A, Azzopardi K, Dahan S, Sircili MP, Franzolin MR, Trabulsi LR, Phillips A,Gomes TAT, Xu J, Robins-Browne R, Frankel G. 2005. Distribution of *tccP* in clinical enterohemorrhagic and enteropathogenic *Escherichia coli* isolates. *J. Clin. Microbiol.* **43**: 5715-5720.

Moreira CG, Palmer K, Whiteley M, Sircilli MP, Trabulsi LR, de Castro AFP, Sperandio V. 2006. Bundle forming pili and EspA are involved in biofilm formation by enteropathogenic *Escherichia coli*. *J. Bacteriol.* **188**: 3952-3961.

Bando SY, Trabulsi LR, Moreira-Filho CA. 2007. Genetic relationship of diarrheagenic *Escherichia coli* pathotypes among the enteropathogenic *Escherichia coli* O serogroup. *Mem. Inst. Oswaldo Cruz*. **102**: 169–174.

Carvalho VM, Irino K, Onuma DL, de Castro AFP. 2007. Random amplification of polymorphic DNA among enteropathogenic *Escherichia coli* isolated from non-human primates and humans. *Braz. J. Med. Biol. Res*. **40**: 237-241.

Aidar-Ugrinovich L, Blanco J, Blanco M, Blanco JE, Leomil L, Dahbi G, Onuma DL, da Silveira WD, de Castro AFP. 2007. Serotypes, virulence genes and intimin types of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) isolated from calves in São Paulo, Brazil. *Int. J. Food Microbiol.* **115**: 297-306.

Rocha LB, Piazza RMF. 2007. Production of Shiga toxin by Shiga toxin expressing *Escherichia coli* (STEC) in broth media: from divergence to definition. *Lett. Appl. Microbiol.* **45**: 411-417.

Bueris V, Sircili MP, Taddei CR, Santos MF, Franzolin MR, Martinez MB, Ferrer SR, Barreto ML, Trabulsi LR. 2007. Detection of diarrheagenic *Escherichia coli* from children with and without diarrhea in Salvador, Bahia, Brazil. *Mem. Inst. Oswaldo Cruz* **7**: 839-44.

Waldir Pereira ELIAS Júnior

Instituto Butantan
Avenida Vital Brazil, 1500 – Butantã
05503-900 – São Paulo, SP – Brasil

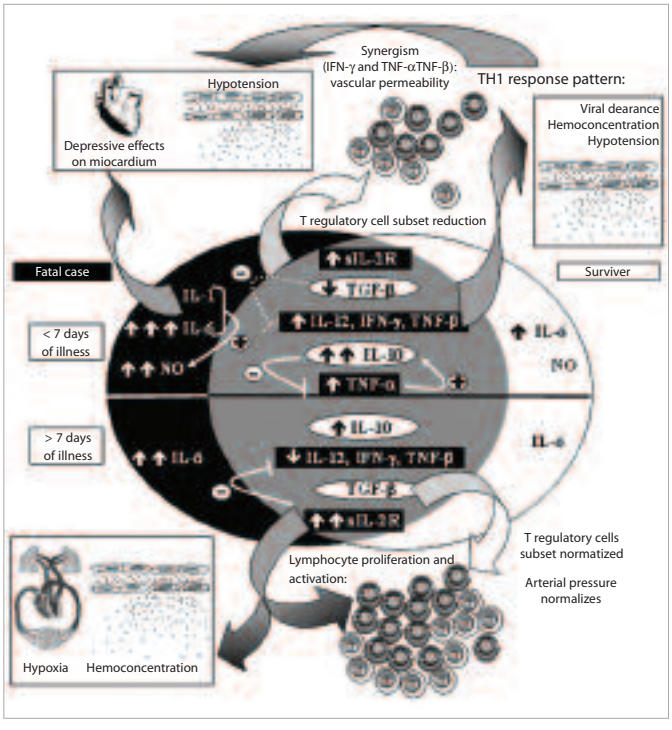
+55-11-3726-7222 r. 2075
wpelias@usp.br

STUDY ON BRAZILIAN ARBOVIRUSES AND RODENT RELATED VIRUSES

Luiz Tadeu Moraes FIGUEIREDO

Ribeirão Preto Medicine School / University of São Paulo (USP)

This thematic research project includes 13 sub-projects on arboviruses and rodent related viruses that cause human diseases in Brazil. This project will be carried out in 4 years. The flavivirus dengue type 1 was introduced in Rio de Janeiro in 1986 and spread in Brazil causing large outbreaks. In 1991, dengue type 2 virus caused an outbreak in Rio de Janeiro where dengue hemorrhagic fever/dengue shock syndrome (dhf/dss) cases were first reported. Dengue became endemic in Brazil with circulation of 2 serotypes in cities infested by *Aedes aegypti*. After 1998, hundreds of thousands of dengue cases have been yearly reported in Brazil and the number dhf/dss cases have increased. In 2001, dengue type 3 virus was introduced in Rio de Janeiro. More than 800,000 dengue cases were reported in 2002 including more than a thousand dhf/dss cases, and about 100 fatalities. The situation of dengue in Brazil is worsening, and it probably will become a serious public health problem as observed in the southeast of Asia. As part of this research project, molecular biology diagnostic methods of dengue, studies on dengue pathogenesis and genomic markers of dengue virus virulence will be studied. Sylvatic yellow fever is expanding in Brazil related to a zoonosis of primates transmitted by haemagoggus mosquitoes. In the past 4 years, dozens of severe human cases of yellow fever, many of them lethal, have been reported among people living close to the highly populated areas of Brasília, Goiânia and Belo Horizonte, as well as in the north of the state of São Paulo. The urbanization of yellow fever threats Brazil, especially in cities infested by the *Aedes aegypti* vector. The attenuated 17dd yellow fever vaccine is highly immunogenic and it is a suitable tool in order to avoid urbanization of the disease. However, some rare fatal cases did occur after vaccination, which could impair an intensive use of the vaccine. As part of this project, the structure and functional mechanism of the ns5 rna polymerase of yellow fever will be studied in order to allow further studies on specific antiviral drugs. The



Schematic model of cytokine participation in HCPS pathogenesis based on results obtained in the present study. The black circle includes findings in fatal cases while the white circle includes findings in survivors. Findings common to both are in intersection (gray area). Cytokines in black boxes are pro-inflammatory whereas cytokines in white boxes are anti-inflammatory. The gray arrows indicate inflammatory effects and their consequences, whereas white narrows indicate anti-inflammatory effects and their consequences.

Orthobunyavirus oropouche is the causative agent of the second Brazilian arboviral disease in number of reported cases. The virus causes outbreaks of acute febrile illness and encephalitis in towns at the borders of rivers of the Amazon region. Oropouche virus is maintained in urban cycles involving *Culicoides paraensis* vector and man. About 500,000 Brazilians were infected by the Oropouche virus in the last decades. We aim to test antiviral drugs against the Oropouche virus; to study a hamster experimental model of Oropouche fever, virus propagation in cell cultures, and mechanisms of virus replication. A cold-adapted virus strain is to be used for creating a vaccine. The rodent related hantaviruses are known to cause cardiopulmonary syndrome (HCPS).

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The Hantavirus Cardiopulmonary Syndrome (HCPS) is an emerging syndrome in the Americas resulting from multiple pathogenic factors associated with intense immune activation and changes in vascular permeability. The aim of this study was to determine the profile of serum cytokines in HCPS patients and their correlation with clinical parameters, severity and outcome of illness. By studying 21 HCPS patients we found that IL-6 probably has a more important role in the pathogenesis of HCPS than TNF-α, being associated with a fatal outcome. We have also shown that the immune response in HCPS follows a TH1 pattern, and that the magnitude of TH1 response effector cytokines is correlated to HCPS severity. The decreased levels of TGF-β observed in HCPS patients suggest that the immunoregulatory activity could be damaged in an early stage of illness. HCPS was first described in Brazil in 1993, and about 240 cases have been reported with a 40% fatality rare. The cases we studied presented prodromic fever that involved after 3 to 5 days to dyspnea, respiratory failure and shock. Thrombocytopenia and elevated hematocrit were also observed in these cases. We have also developed an RT-PCR diagnostic method for hantavirus, and detected the virus genome in the blood of 11 hcps patients. Nucleotide sequences of RT-PCR amplicons from these patients showed a 96.5 to 87.7% homology with the Araraquara hantavirus genome, thus showing that these cases were caused by this virus. We also performed a serologic survey for hantavirus in 2001 in Jardinópolis, São Paulo State, including 818 participants, and 14.3% of them presented IgG antibodies against the Andes hantavirus as detected by ELISA. Soropositive participants were not associated to sex, age, previous contact with rodents or severe pneumonia. These results suggest that hantaviruses may be causing undiagnosed asymptomatic or clinically minor infections in Brazil. It entails important questions such as whether more than one hantavirus strain would be circulating in Jardinópolis, causing mostly benign infections. Could hcps be associated with some predisposing condition in the infected individuals? As part of this project, a search for benign infections will be carried out in order to study the disease and the infecting hantavirus. Virus-reli interactions and the pathogenesis of HCPS will be studied based on the detection of cytokines and genetic markers. Additionally, a recombinant N protein of the Araraquara virus will be produced for diagnostic methods and as a vaccine candidate.

MAIN PUBLICATIONS

Bronzoni RVM, Nogueira RMR, Nunes M, Figueiredo LTM. 2005. Detection and identification of Brazilian alphaviruses and flaviviruses by multiplex RT-PCR. *Journal of Clinical Microbiology*. **43**:696-702.

Borges AAB, Campos GM, Moreli ML, Souza RLM, Figueiredo LTM. 2006. Pathogenesis of Hantavirus Cardiopulmonary Syndrome and the human immune responses to Hantavirus. *Microbes & Infection*. **8**:2324-30.

Aquino VH, Anatriello E, Silva EV, Vasconcelos PFC, Vieira DS, Batista WC, Bobadilla ML, Vasquez C, Moran M, Figueiredo LTM. 2006. Molecular epidemiology of dengue type 3 virus in Brazil and Paraguay. *American Journal of Tropical Medicine and Hygiene*. **75**:910-5.

Livonesi MC, Sousa RLM, Badra SJ, Figueiredo LTM. 2006. Ribavirin action on Brazilian Orthobunyavirus. *American J. of Tropical Med. and Hygiene*. **75(5)**:1011-6.

Mendoza LP, Bronzoni RM, Takayanagui OM, Aquino VH, Figueiredo LTM. 2007. Viral infections of the central nervous system in Brazil. *Journal of Infection*. **54**:589-96.

Mondini A, Bronzoni RVM, Cardeal ILS, dos Santos TMIL, Lázaro E, Nunes SHP, Silva GCD, Madrid MCFS, Rahal P, Figueiredo LTM, Chiaravalloti-Neto F, Nogueira ML. 2007. Simultaneous infection by DENV-3 and SLEV in Brazil. *Journal of Clinical Virology*. **40**:84-6.

Livonesi MC, Sousa RLM, Badra SJ, Figueiredo LTM. 2007. Mycophenolic Acid action on Brazilian *Orthobunyavirus*. *Intervirology*. **50**:204-8.

Livonese MC, de Sousa RLM, Badra SJ, Figueiredo LTM. 2007. *In vitro* and *In vivo* studies of the Interferon-Alpha action on distinct Orthobunyavirus. *Antiviral Research*. **74**:121-8.

Saggioro FP, Rossi MA, Duarte MIS, Martin CCS, Alves VAF, Moreli ML,Figueiredo LTM, Moreira JE, Borges AA, Neder L. 2007. Hantavirus infection induces a typical myocarditis that may be responsible for myocardial depression and shock in Hantavirus Pulmonary Syndrome. *Journal of Infectious Diseases*. **145**:1541-9.

Borges AA, Figueiredo LTM. 2008. Mechanisms of shock in Hantavirus Pulmonary Syndrome. *Current Opinion in Infectious Diseases*. (in press).

Luiz Tadeu Moraes FIGUEIREDO

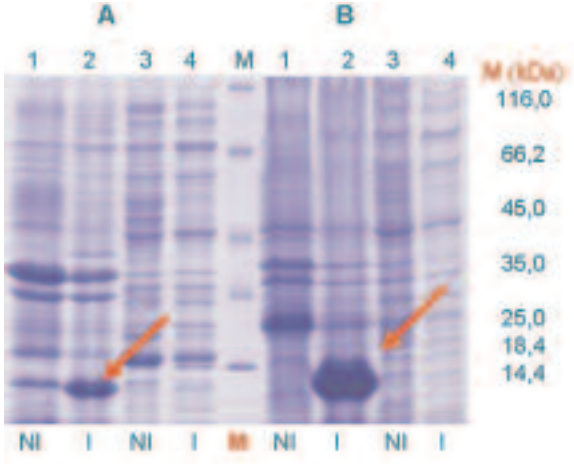
Faculdade de Medicina de Ribeirão Preto
Universidade de São Paulo (USP)
Avenida Bandeirantes, 3900 – Monte Alegre
CEP 14048-900 – Ribeirão Preto, SP – Brasil

+55-16-602-3271
ltmfigue@fmrp.usp.br

CLONING AND EXPRESSION OF PROTEINS IDENTIFIED IN THE GENOME OF *Leptospira interrogans* serovar *Copenhageni* FOR POTENCIAL VACCINE AND DIAGNOSTIC USE

Paulo Lee HO

Butantan Institute

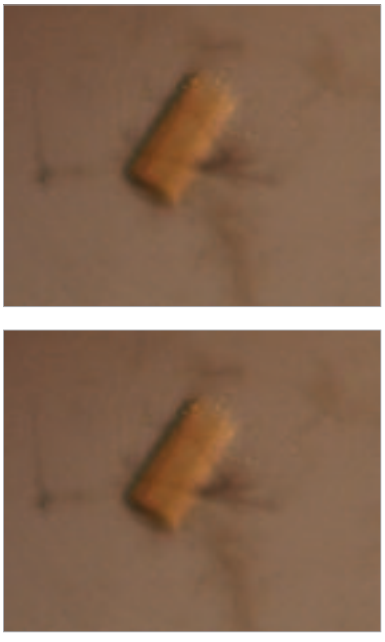


Purification of recombinant proteins. SDS-PAGE (15%) of purified recombinant proteins obtained by metal affinity chromatography. Proteins encoded by (1) LIC10009, (2) LIC10191, (3) LIC11947, (4) LIC12730, (5) LIC10494, (6) LIC12906

Leptospirosis is considered the most widespread zoonotic disease caused by the spirochaete from the *Leptospira* genus. In Brazil, 80% of all the diagnosed cases are due to *Leptospira interrogans* serovar *Copenhageni*. Rats are the main reservoir in urban centers and the bacteria are disseminated through animal urines. Around 10,000 cases of leptospirosis are annually notified to FUNASA (Health Ministry, Brazil), epidemically associated to the rainy seasons (http://www.funasa.gov.br). A promising way to treat this health problem would be a vaccination program, since the control of rats is a difficult task. Thus, the development of a suitable vaccine against leptospirosis is of seminal importance, since up to date, there is no licensed human vaccine against leptospirosis. Furthermore, the general clinical signs of this disease are often confounded with those of other diseases, like dengue and flu symptoms, reinforcing the need for an efficient diagnose assay for leptospirosis, not yet available in the market. Recently, the genome of the *Leptospira interrogans* serovar *Copenhageni* was described. The information of the genome would help to identify potential *Leptospira* vaccine antigen candidates or antigens for diagnose applications. The present project is divided in 3 subprojects, aiming: 1) cloning of 8 selected hemolysins. Recombinant proteins will be expressed in *E. coli* and further evaluated for their potential use as vaccine antigens and for potential use in diagnose. Biochemical and immunological characterization will also be performed; 2) cloning of 5 selected lipoproteins. Recombinant proteins will be expressed in *E. coli* and further evaluated for their potential use as vaccine antigens and for potential use in diagnose. Biochemical and immunological characterization will also be performed; 3) cloning and expression of 5 of the above antigens in *Salmonella thyphimurium*. The recombinant *Salmonella* will be evaluated as live vaccine against *Leptospira*.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We have accumulated data from dozens of potential antigens of *Leptospira*. Though we have initially proposed the study of 8 hemolysins and 10 lipoproteins, being 10 of them also expressed as live vaccine in *Salmonella thyphimurium*, we have expanded this universe. For instance, in the case of the subproject dealing with hemolysins, we started with 8 ORFs, but we ended up with 20 ORFs. All these were cloned and evaluated for expression in *E. coli*. This represents our first step in our experimental approach to uncover new antigens for vaccine or diagnostic kit development for leptospirosis. If these ORFs were expressed in *E. coli*, the study progress to step 2. If not, we try to express them using other *E. coli* strains, conditions. If they are still not expressed, we look for new ORFs to replace them. For those that we were able to express, the proteins are purified and antiserum is produced against each of them. These sera are immunocharacterized and used to screen a panel of different *Leptospira* serovars to define if they are conserved antigens among them. Those that are conserved, they are selected for the next steps, since we are searching for antigens that would compose a universal vaccine against all the *Leptospira* serovars but not a vaccine against a specific serovar. These ORFs are biologically characterized, for reactivity against patient sera as well as assayed as protective antigens in a hamster model of immunization and challenge with pathogenic virulent *Leptospira*. At least one antigen we presented in *Salmonella* was able to elicit partial protection in animal model. We were able to identify several conserved antigens among the serovars that would be useful for leptospira diagnosis, new adhesins that would mediate leptospira attachment to host cells, new antigens that mediate the inflammatory response observed during leptospira infection, besides other findings. We can anticipate that the project will at least increase our comprehension of this bacteria and the disease it causes that will allow us in the future to interfere with the progress of the disease.



Leptospira *antigen crystals for determining three-dimensional structures*

MAIN PUBLICATIONS

Hauk P, Negrotto S, Romero EC, Vasconcellos SA, et al. 2005. Expression and characterization of HlyX hemolysin from *Leptospira interrogans* serovar *Copenhageni*: Potentiation of hemolytic activity by LipL32. *Biochemical and Biophysical Research Communication*. **333**:1341-1347.

Barbosa AS, Abreu PA, Neves FO, Atzingen MV, et al. 2006. A newly identified leptospiral adhesin mediates attachment to laminin. *Infect Immun*. **74**: 6356-6364.

Nascimento ALTO. 2006. ?Leptospira interrogans: Genomics and "Immunomics" in Molecular Biology of Spirochetes (Cabello FC, Hulinska D, Godfrey HP, eds), **373**:115-123. *NATO Science Series: Life and Behavioural Sciences* ,416pp. IOSPress, ISBN: 1-58603-665-3.

Neves FO, Abreu PA, Vasconcellos SA, de Morais ZM, Romero EC, Nascimento ALTO. 2007. Identification of a novel potential antigen for early-phase serodiagnosis of leptospirosis. *Arch Microbiol*. **188**: 523-532.

Vieira ML, D'Atri LP, Schattner M, Habarta AM, et al. 2007. A novel leptospiral protein increases ICAM-1 and E-selectin expression in human umbilical vein endothelial cells. *FEMS Microbiol Lett*. **276**:172-180.

Hauk P, Macedo F, Romero EC, Vasconcellos SA, Morais ZM, Barbosa AS, Ho PL. 2008. LipL32, the Major Leptospiral Lipoprotein: the C-terminus is the Primary Immunogenic Domain and Mediates Interaction with Collagen IV and Plasma Fibronectin. *Infection and Immunity*, in press.

Atzingen MV, Barbosa AS, De Brito T, Vasconcellos SA, Morais ZM, Lima DCM, Abreu PAE, Nascimento ALTO. 2008. Lsa21, a novel leptospiral protein binding adhesive matrix molecules and present during human infection, submitted.

Gomez RM, Vieira ML, Schattner M, Malaver E, Watanabe MM, et al. 2008. Outer membrane proteins of Leptospira interrogans stimulate human umbilical vein endothelial cells (HUVECS) and express during infection, submitted.

Neves FO, Abreu PAE, Nascimento ALTO. 2006. Patent application pending. "Processo de clonagem, expressão e purificação de proteína de *Leptospira* sp para diagnostico", processo PI 0602669-9.

Paulo Lee HO

Instituto Butantan
Centro de Biotecnologia
Avenida Vital Brasil, 1500 – Butantã
05503-900 – São Paulo, SP – Brasil

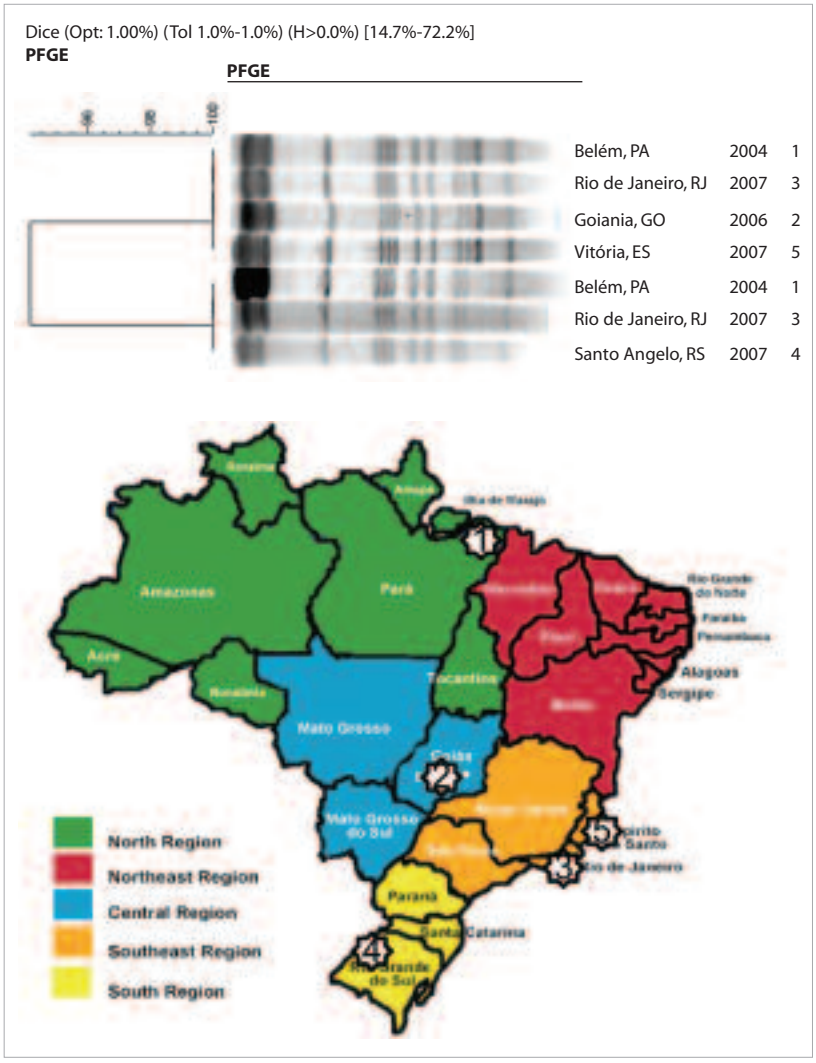
+55-11-3726-7222 r. 2244
hoplee@butantan.gov.br

MYCOBACTERIA OF CLINICAL SIGNIFICANCE IN BRAZIL: MOLECULAR CHARACTERIZATION, INTERACTION WITH THE ENVIRONMENT AND WITH MACROPHAGES

Sylvia Luisa Pincherle Cardoso LEÃO

Microbiology, Immunology and Parasitology Departament / Federal University of São Paulo (Unifesp)

Non-tuberculous micobacteria (NTM) are emerging pathogens, with potential to cause infections in immunocompromized and also immunocompetent patients, frequently as a complication of invasive procedures, like ophthalmologic, plastic, and laparoscopic surgeries, cosmetic procedures, and endoscopically based exams. Studies that improve our knowledge about the biology of these bacteria and the interaction with the environment and host cells will be useful for future elaboration of control programs and therapeutic approaches. In sub-project 1, three molecular methods will be used to type isolates from an outbreak of infections after surgical procedures that occurred at Belém (PA) between 2004 and 2005. The results will be compared with patients' data for analysis of epidemiological correlation. A bank of genetic profiles will be organized and a fluxogram will be elaborated to help in the management of future outbreaks. In sub-project 2, we will investigate the presence of plasmids and mycobacteriophages in outbreak and environment isolates and their role in horizontal transfer of genetic information. In sub-project 3, the potential of these bacteria to produce and to survive in biofilms will be evaluated and the role of these structures in resistance to biocides commonly used for equipment disinfection will be investigated. In sub-project 4, the interaction of a *M. avium* virulent isolate with epithelioid-like cells (recombinant interleucine-4 treated murine peritoneal macrophages), constituting an *in vitro* model developed by our group, will be investigated to improve our understanding of the functions of epithelioid cells present in granuloma.



*PFGE patterns of representative isolates from each outbreak (Belém, Goiânia, Rio de Janeiro, Santo Ângelo, and Vitória). Two PFGE patterns, * and **, differing in one band, were identified in outbreak isolates. Columns indicate the city, year of the outbreak, and map location, respectively.*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We are presently involved in collaborative projects to extend the molecular studies and also to determine the susceptibility to antimicrobial agents of a significant subset of *Mycobacterium massiliense* isolates. An outbreak of infections affecting 311 patients submitted to different invasive procedures occurred in 2004-2005, at the city of Belém, in the North region of Brazil. In 2007, similar cases were detected in other States from Brazil – Goiás, Rio de Janeiro, Rio Grande do Sul, Espírito Santo and Paraná. A subset of isolates from these sites was compared to the Belém isolates and results of DNA sequencing and PFGE confirmed, unexpectedly, that the same *M. massiliense* strain was responsible for the outbreaks in the different States.

In a previous project we have detected by PCR the presence of a *Mycobacterium avium* specific insertion sequence, IS1245, in colonies of *Mycobacterium kansasii*. Both species were isolated from a bone marrow sample from the same HIV-positive patient. The presence of this element in isolated colonies of *M. kansasii* was confirmed by Restriction Fragment Length Polymorphism (RFLP) using a probe complementary to the IS1245. A plasmid was detected by PFGE in colonies of *M. avium* and *M. kansasii* which generate amplicons by PCR-IS1245 while colonies of *M. kansasii* that did not generate amplicons by PCR-IS1245 did not bear this plasmid. These results opened new perspectives for this project, and we are engaged in the characterization of this plasmid and the study of conjugative mechanisms in mycobacteria. There are few studies about horizontal gene transfer in mycobacteria and results obtained with this project will boost up the understanding of mechanisms of gene acquisition and its influence in virulence, evolution and genetic diversity in mycobacteria.

Our group succeeded in the obtention of an *in vitro* model treatment, during 7 days, of mouse resident peritoneal macrophages with r-IL-4) for the generation of epithelioid-like cells (ECs-like) that can be used as a surrogate of epithelioid cells (ECs) found in granulomas. We are presently studying cell signaling in mouse peritoneal macrophages upon rIL-4 treatment, before the performance of experiments with infected cells. Experiments to obtain *M. avium* expressing green fluorescent protein are under way and the recombinant bacteria will be used in trafficking experiments.

Rough and smooth variants from one isolate from the Belém outbreak were separated and these phenotypes were shown to be stable after ten passages in solid medium. Histopathological analyses of the excised spleens are under way. *Ex vivo* experiments to study the infection of ECs-like and macrophages with the two variants will be also performed to check how these cells cope with the infection. The results will be compared to those obtained with environmental isolates of the same species.

MAIN PUBLICATIONS

Marcondes AG, Shikama MLM, Vasconcellos SA, et al. 2006. Comparação entre a técnica de cultivo em camada delgada de ágar Middlebrook 7H11 e meio de Stonebrink para isolamento de *Mycobacterium bovis* em amostras de campo. *Braz. J. vet. Res. anim. Sci*. **43(3)**:362-369.

Oliveira EMD, Rodrigues CAR, Leão SC, Amaku M, Ferreira-Neto JS. 2006. Estudo da dinâmica da infecção por *Mycobacterium avium* em uma população suína através de modelagem matemática. *Arq. Inst. Bio.* **73(4)**:409-414.

Padoveze MC, Fortaleza CMCB, Freire MP, Assis DB, et al. 2007. Outbreak of surgical infection caused by non-tuberculous mycobacteria in breast implants in Brazil. *J Hosp Infect*. **67(2)**:161-167.

Martin A, Uwizeye C, Fissette K, De Rijk P, et al. 2007. Application of the hsp65 PRA method for the rapid identification of mycobacteria isolated from clinical samples in Belgium. *J. Microbiol. Methods*. **71(1)**:39-43. Epub 2007 Aug 1.

Leão SC, Viana-Niero C. 2007. Surtos de infecções por micobactérias após procedimentos cirúrgicos. *Boletim da Urologia*, **4**:103-104.

Campos ACE, Molognoni F, Melo FHM, Galdieri LC, et al. Oxidative stress modulates DNA methylation during melanocyte anchorage blockade. *Neoplasia*. Accepted, 2007.

Viana-Niero C, Lima CVB, Lopes ML, Rabello MCS, et al. 2008. Molecular characterization of *Mycobacterium massiliense* and *Mycobacterium bolletii* in outbreaks of infections after laparoscopic surgeries and cosmetic procedures. *J. Clin Microbiol.* **46(3)**:850-855.

Chimara E, Ferrazoli L, Ueky SYM, Martins MC, et al. 2008. Reliable identification of mycobacterial species by PCR-Restriction Enzyme Analysis (PRA)-hsp65 in a reference laboratory and elaboration of a sequence-based extended algorithm of PRA-hsp65 patterns. *BCM Microbiology*. **8**:48.

Moraes PRS, Chimara E, Telles MAS, Ueki SYM, et al. Identification of non-tuberculous mycobacteria from the Central Public Health Laboratory from Mato Grosso do Sul and analysis of clinical relevance. *Braz. J. Microbiol*. (in press).

Sylvia Luisa Pincherle Cardoso LEÃO

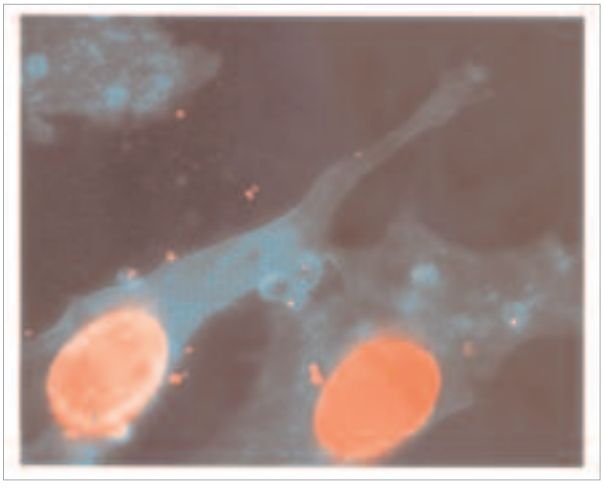
Universidade Federal de São Paulo (Unifesp)
Depto de Microbiologia, Imunologia e Parasitologia
Rua Botucatu, 862, 3º andar – Vila Clementino
04023-062 – São Paulo, SP – Brasil

+55-11-5576-4537
sylvia@ecb.epm.br

MOLECULAR STUDIES ON *Trypanosoma cruzi* AND ITS INTERACTION WITH CELLS AND FACTORS FROM THE HOST *IN VITRO* AND *IN VIVO*

José Franco da SILVEIRA Filho

Microbiology, Immunology and Parasitology Departament / Federal University of São Paulo (Unifesp)



Trypanosoma cruzi *amastigotes recruit lipid raft marker GM1 during host cell invasion*

Trypanosoma cruzi, the etiological agent of Chagas' disease, is a highly relevant microorganism from both medical and biological points of view. Although vectorial transmission is mostly under control in the Southern Cone, *T. cruzi* transmission still prevails in Latin America where 15-16 million people are infected. Outbreaks of acute cases of Chagas' disease by oral infection have been reported in different areas of Brazil. Our group has extensively studied host cell invasion by trypomastigotes and amastigotes, an essential step in the establishment of infection in man and other vertebrate hosts. Oral infection has become the focus of our studies in the last years. The mechanisms of *in vitro* and *in vivo T. cruzi* infection are beginning to be unveiled. Parasite molecules involved in host cell interactions are being analyzed regarding their expression in different developmental forms of *T. cruzi* strains, and also regarding their genomic organization and structure.

We intend to study the interaction of *T. cruzi* with host cells and factors both *in vitro* and *in vivo*. The project involves the analysis of molecules previously identified by our group as well as the identification and characterization of new molecular and cellular components. Among our objectives is the identification of host cell receptors for parasite surface proteins, and the study of the intracellular traffic of *T. cruzi* infective forms. We plan to use host cells transfected with different genes as well as co-infection models with other trypanosomes and invasive bacteria.

Concerning the metacyclic surface antigens, we intend to identify transcribed and translated genes by hybridizations with DNA microarrays and partial sequencing of proteins with mass spectroscopy, respectively. Recombination mechanisms responsible for the generation of variants in these multigenic families will be studied in parasites transfected with artificial and specially designed *T. cruzi* chromosomes. These studies will bring new information on the mechanisms controlling gene expression in this parasite and on the evolution of multigenic families.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Host cell invasion by *T. cruzi* is a complex process in which various host as well as parasite components interact, triggering the activation of signaling cascades and calcium mobilization in both cells. Our studies with metacyclic trypomastigotes from different strains have elucidated the mechanisms of parasite entry into target cells.

Outbreaks of severe acute Chagas' disease, acquired by oral route, have been frequently notified in Brazil. We have investigated in the murine model the molecular basis of oral *T. cruzi* infection using an isolate derived from a patient with severe clinical manifestations, upon ingestion of contaminated sugar cane juice. The role of metacyclic surface glycoproteins in oral infection was established.

We found that recombinant GP82 protein is able to inhibit the development of murine melanoma cells. When injected with GP82 in the tumor area, the mice that had received tumorigenic cells developed melanoma at significantly lower pace and survived longer, as compared to control.

Studies on *T. cruzi*-host interactions revealed unique features of amastigote expression that appeared to be host-dependent. In the double-infection model using *Vero cells* harboring the bacterium *C. burnetii* we observed strain and infective form dependences in both invasion and trafficking properties. Studies aiming at the identification of membrane components that might be involved in host-parasite interactions revealed for the first time that host cell cholesterol and membrane lipid raft components are involved in both trypomastigote and amastigote invasion of mammalian cells. This work was honored with the cover of *International Journal Parasitology*.

Analysis of the expression of gp82 and gp90 genes in the digestive tract of the insect vector *R. prolixus* showed that the stabilization of these mRNAs is linked with their translation. We identified gp82 alleles lacking the motifs for adhesion of the parasite to mammalian cells.

Computer models were used to estimate the variation generation capacity of surface gene families. We found that genes relevant to host-parasite interactions exhibited high volatility (anti-robust pattern), which may be related to the capacity of the parasite to evade the host immune system. Measures of genetic robustness may detect variations between potential drug targets at the protein level. The simulations showed that nuclear genes tend to be relatively more robust against random, multiple-point mutations than surface protein genes.

MAIN PUBLICATIONS

Atayde VD, Cortez M, Souza R, Franco da Silveira J, Yoshida N. 2007. Expression and cellular localization of molecules of gp82 family in *Trypanosoma cruzi* metacyclic trypomastigotes. *Infection and Immunity.* **75**: 3264-3270.

Atayde VD, Jasiulionis M, Cortez M, Yoshida N. 2008. A recombinant protein based on *Trypanosoma cruzi* surface molecule gp82 induces apoptotic cell death in melanoma cells. *Melanoma Research*. In Press.

Azuaje F, Ramirez JL, Da Silveira JF. 2007a. An exploration of the genetic robustness landscape of surface protein families in the human protozoan parasite *Trypanosoma cruzi*. *IEEE Transactions on Nanobioscience*. **6(3)**:223-8.

Azuaje F, Ramirez JL, Da Silveira JF. 2007b. In silico, biologically-inspired modelling of genomic variation generation in surface proteins of *Trypanosoma cruzi*. *Kinetoplastid Biology and Disease*, **10(6)**:6.

Coimbra VC, Yamamoto D, KhusAI KG, et al. 2007. Enucelated L929 cells support invasion, differentiation and multiplication of *Trypanosoma cruzi* parasites. *Infection and Immunity.* **75**: 3700-3706.

Cordero E, Gentil LG, Crisante G, Ramirez JL, Yoshida N, Añez N, Franco da Silveira J. 2008. Expression of GP82 and GP90 surface glycoprotein genes of *Trypanosoma cruzi* during *in vivo* metacyclogenesis in *Rhodnius prolixus*. *Acta tropica.* **105**: 87-91.

Covarrubias C, Cortez M, Ferreira D, Yoshida N. 2007. Interaction with host factors exacerbate *Trypanosoma cruzi* cell invasion capacity upon oral infection. *International Journal for Parasitology.* **37**: 1609-1616.

Fernandes MC, L'Abbate C, Andreoli WK, Mortara RA. 2007a. *Trypanosoma cruzi* cell invasion and traffic: influence of *Coxiella burnetii* and pH in a comparative study between distinct infective forms. *Microbial Pathogenesis*. **43**: 22-36.

Fernandez MCD, Cortez M, Yoneyama KA, Straus AH, Yoshida N, Mortara RA. 2007b. Novel strategy in *Trypanosoma cruzi* cell invasion: implication of cholesterol and host cell microdomains. *International Journal for Parasitology.* **37**:1431-1441.

José Franco da SILVEIRA Filho

Universidade Federal de São Paulo (Unifesp)
Departamento de Microbiologia,
Imunobiologia e Parasitologia
Rua Botucatu, 862 – 6º andar – Vila Clementino
04023-062 - Sao Paulo, SP – Brasil

+55-11-5576-4532 ext. 28
franco@ecb.epm.br