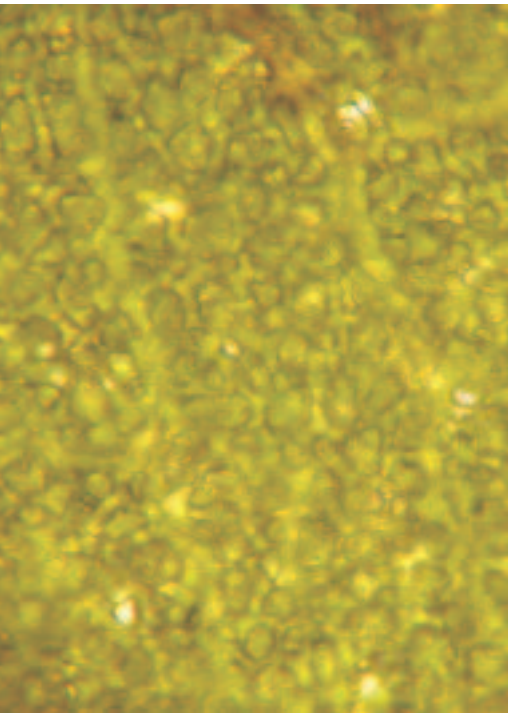
THEMATIC PROJECTS

FAPESP
THE STATE OF SÃO PAULO
RESEARCH FOUNDATION

OPPORTUNITIES FOR HEALTH RESEARCH IN BRAZIL

MEDICINE

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.

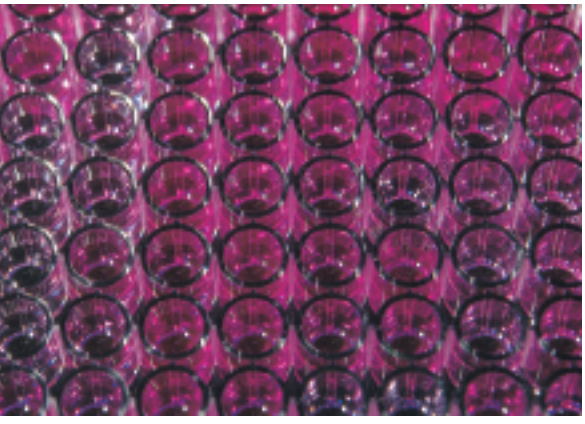
SCIENTIFIC OPPORTUNITIES IN SÃO PAULO, BRAZIL

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.

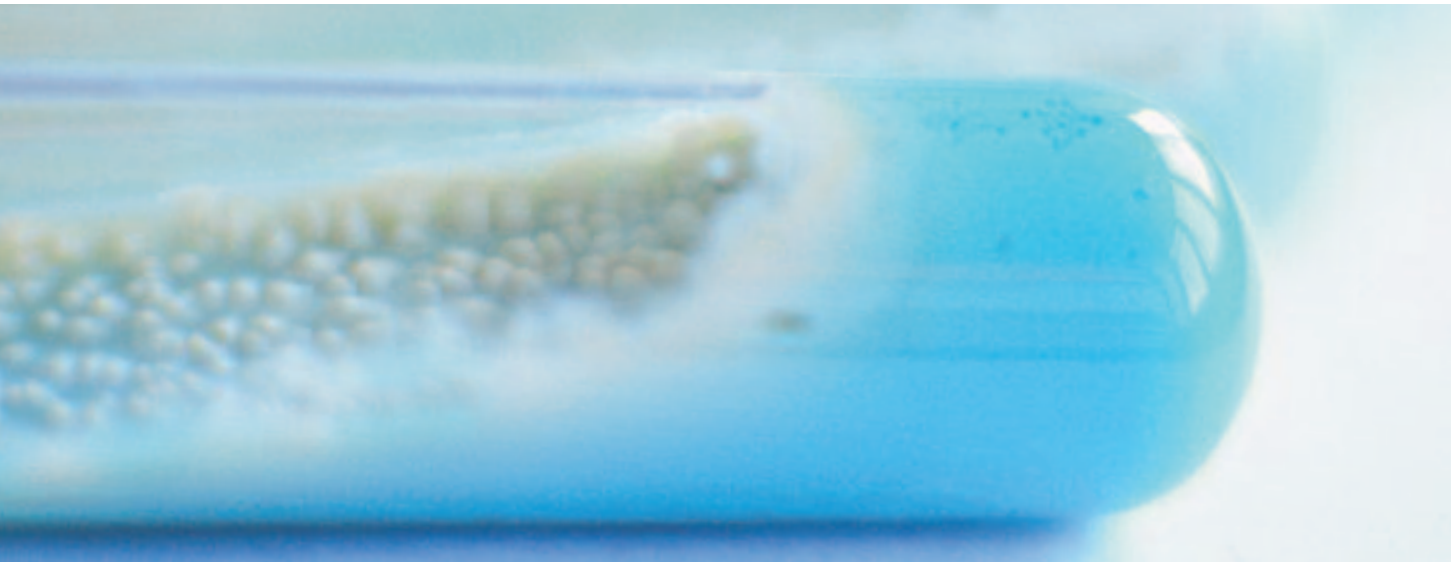
In 2008 FAPESP will invest US$ 388 million in fellowships and research grants. The success rate for proposals in the fellowship programs ranges from 40 per cent to 63 per cent. In the grants programs the proposal success rate ranges from 40 per cent to 60 per cent, depending on the particular type of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

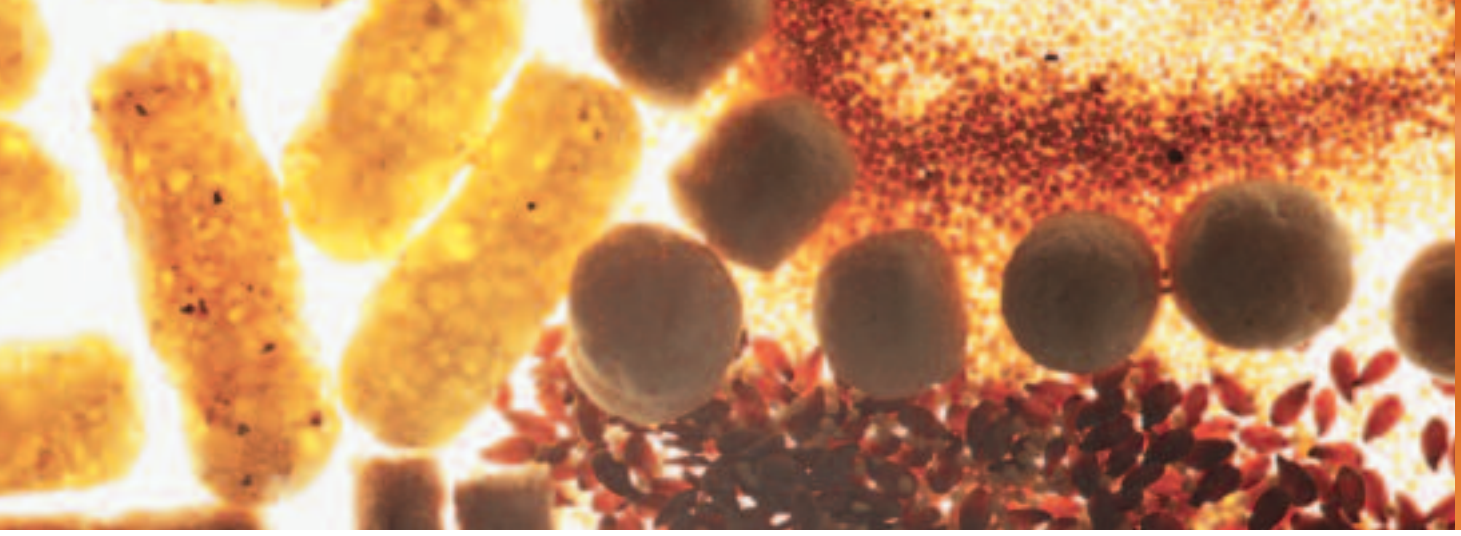
OPPORTUNITIES AND CHALLENGES

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.





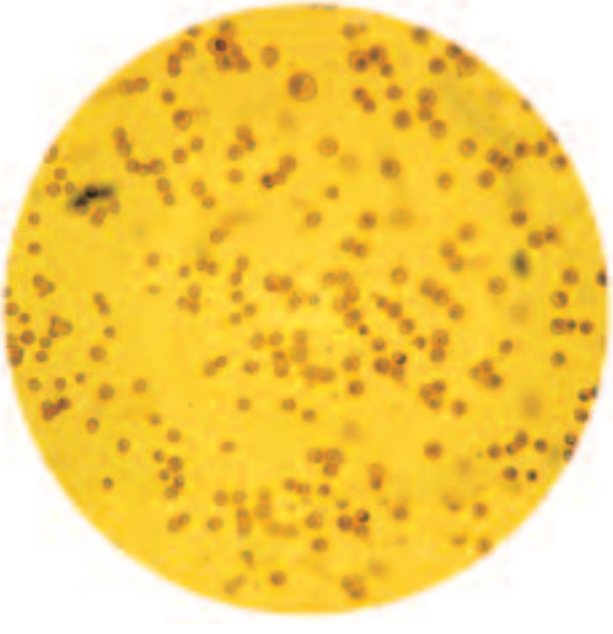
RESULTS OF GREAT IMPACT

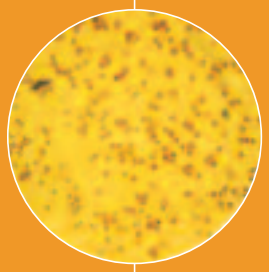
When the research program for Thematic Projects was created, in 1990, FAPESP's objective was to provide a qualitative leap in Brazilian scientific research and meet the state of São Paulo's own particular demands for development. Since then, 1,100 projects in all fields of knowledge have been selected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

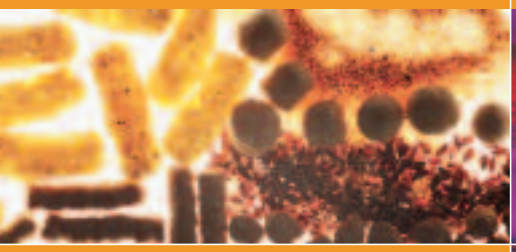
Thematic Projects are characterized by the breadth of their research and the boldness of their objectives. They are supported for four years (as opposed to two years for a regular research grant) and are lead by teams of experienced researchers.

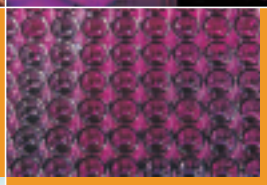
Thematic Projects are funded, on the average, with 450 thousand dollars, plus fellowships. The salaries for the investigators and staff are not included in this amount since in Brazil they are paid by their universities. Each project is lead by 3 PI's and involves several undergraduate and graduate students.

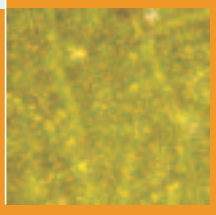
Thematic Projects create opportunities for scientists in São Paulo to advance knowledge by creating internationally competitive science, while, simultaneously, educating a new generation of researchers.











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AN EPIDEMIOLOGICAL STUDY OF PSYCHIATRIC DISORDERS IN THE SÃO PAULO METROPOLITAN REGION: PREVALENCE, RISK FACTORS, AND SOCIAL AND ECONOMICAL BURDEN

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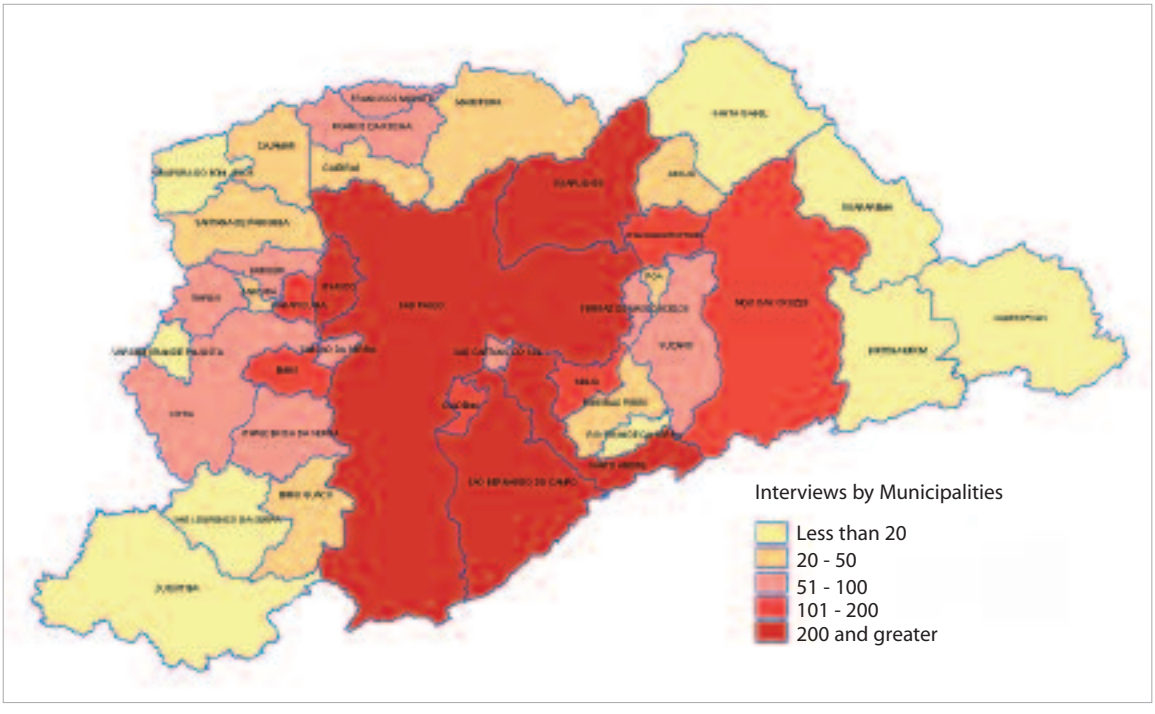

The Região Metropolitana de São Paulo holds 39 municipalities in total, with around 19,7 million inhabiants (2006), which makes it the fifth most populous metropolitan area in the world – Map depicting interviews conducted across municipalities

The Epidemiological Study of Psychiatric Disorders in São Paulo Metropolitan Region ("São Paulo Megacity: Pesquisa sobre Saúde, Bem-Estar e Estresse") aims at identifying the prevalence rates of psychiatric disorders, evaluating the degree of disability associated to them, studying their natural history, and determining their correlates in the adult population dwelling in the São Paulo Metropolitan Region. The main goal will be to provide scientific evidence to direct the implementation of new preventive and therapeutic strategies, as well as to guide the development of health policies which are more suitable to the region reality, and to help in planning health services based on the population needs. This is part of a study that is being conducted in 28 countries coordinated by the World Health Organization, named The World Mental Health Survey.

The relevance of this study is emphasized by "The Global Burden of Disease" project, which demonstrated that the mental disorders and the use of alcohol and other substances are among the main causes of global burden, measured by the number of years lived with disabilities and the number of years lost by premature death, as a consequence of disease. This study will provide data relating to the direct and indirect costs of mental disorders in a Brazilian population and will allow for the proposal of new hypotheses as to the etiology and determinants of mental disorders.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Altogether, 5,237 individuals aged 18 and over, a probabilistic sample of the general population resident in the 39 Municipalities of the São Paulo Metropolitan Region, were interviewed. The questionnaire, applied by trained lay interviewers, was WHO-CIDI PAPI V 5, translated into Portuguese and adapted to the Brazilian reality. Composed of 4,551 variables related to symptoms and risk factors for the mental disorders assessed, it is possible with this instrument to generate diagnosis according to CID-10 and DSMIV psychiatric classifications.

The data collection was completed, and we are in the process of elaborating the sample weights. Preliminary statistical analyses indicated that, from the total sample, 44.4% showed at least one lifetime psychiatric diagnosis. The most frequent diagnoses were depression (22.8%) and anxiety disorders (17%). Use of alcohol and other substances are more frequent in men (78%). The explosive intermittent disorder showed the same distribution between genders and, in general, the anxiety and depressive disorders affect women in a larger proportion.

The São Paulo Megacity was designed to study the multiple aspects of mental disorders, both related to the individuals and to their social context. As a population based study, it will be possible to identify the multiple manifestations of psychiatric syndromes in various levels of severity. We will be able to study the natural history of psychopathology, since we have information about the age of onset of the disorders, the course and the outcome, and it will also be possible to obtain the distribution of psychopathological dimensions. Moreover, it will be possible to evaluate temporal variations, comparing the current results to the study conducted ten years ago in two neighborhoods in the capital of São Paulo (São Paulo Epidemiological Catchment Area Study-ECA-SP).

MAIN PUBLICATIONS

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PAPERS

Andrade LH, Viana MC. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the São Paulo Megacity Survey, in preparation.

Viana MC,Andrade LH. Prevalence, Severity and Comorbidity of 12-Month DSM-IV Disorders in the São Paulo Metropolitan Area: Results from the São Paulo Megacity Survey, in preparation.

Viana MC, Andrade LH. Twelve-month and lifetime use of health services due to mental disorders in the São Paulo Metropolitan Area: Results from the São Paulo Megacity Survey, in preparation.

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STUDY OF THE ALTERATIONS RESULTING FROM THE MALIGNANT TRANSFORMATION OF PLEOMORPHIC ADENOMA INTO CARCINOMA EX-PLEOMORPHIC ADENOMA

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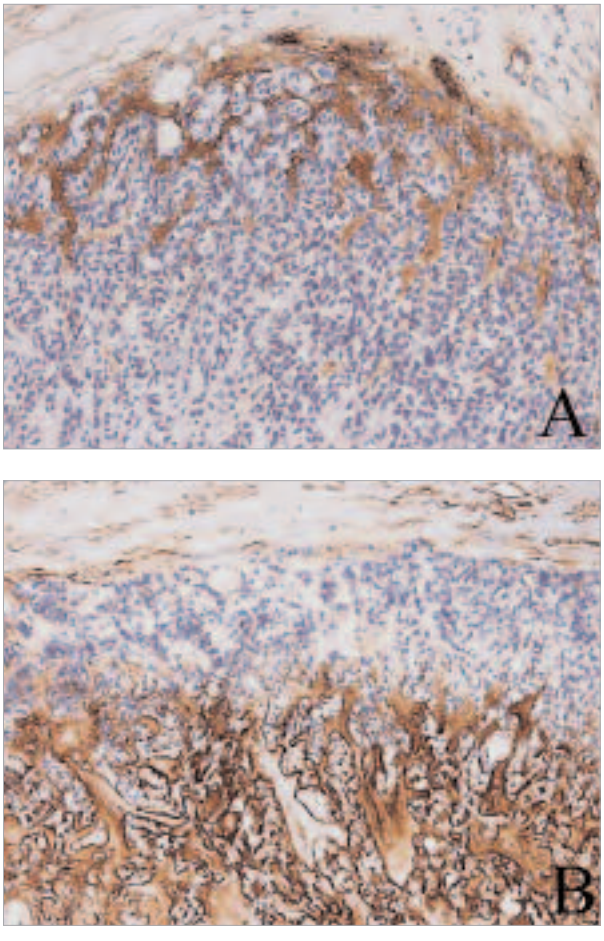

CXPA minimally invasive type, with epithelial component. A and B: different expression of tenascin and fibronectin. Tenascin is expressed only in the invasion front (A) meanwhile, fibronectin is expressed only in the deep area (B) of the same specimen

Carcinoma ex-pleomorphic adenoma (CXAP) is a salivary gland tumor resulting from a malignant transformation of pleomorphic adenoma, usually recurrent or of long duration. It accounts for 5 to10% of all salivary gland tumors. It is considered a high grade tumor with frequent metastasis and death. Since it is a rare malignant transformation of a benign tumor, the CXAP is an interesting model for studying the events that take place during the malignant transformation, such as the acquired metastasis, the alteration of cellular fenotype, the modification of the environment, the occurrence of anti-invasive and invasive components and the chromossome alteration related to the material loss and gain. The aim of this project is to study the CXPA according to the following subjects: i) immunohistochemical profile of tumoral cells in relation to cytoskeleton proteins, adhesion proteins (cadherin and catenin),inhibitor of protease (Maspin);
ii) immunohistochemical profile of tumoral stroma;
iii) study of tumor oncogenes and suppressor genes;
iv) analysis of genetic alterations by comparative genomic hybridization. All the results will be analyzed by comparing the benign area with the malignant area and, when it exists, with the transitional area.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The study of CXPA in its different phases of malignant progression allowed comparative studies between benign and malignant area, including transitional area, contributing to the knowledge of this tumor oncogenesis. The cytoskeletal protein analysis has classified CXPA in tumors with epithelial component and epithelial and/or mioepithelial and highlighted the *in situ* conditions. This way we have contested the literature which considers intracapsular tumor as *in situ* tumor. It has been shown that benign myoepithelial cells surrounding malignant luminal cells of *in situ* areas become more differentiated and produce important proteins related to the tumor suppressor role. It has been observed that maspin was present in the myoepithelial cell possibly as a suppressor tumor preventing tumor growth and metastasis as well as the invasion and motility. P53 protein and C-ERB-2 were observed even in the early phase of malignant transformation contributing to the differential diagnosis between atypical cells without oncogenic potential and carcinomatous group of cells. Concerning tumoral stroma it has been observed that tenascin and collagen type I of fibrilar pattern probably highlight the real invasive front of the tumor. Desmoplasia increases with the tumor progression, being rare at CXPA presenting myoepithelial component. About vascularization, it was observed that the lymphatic vessels were the ones previously present in the benign tumor and that neoangio genesis increased during adenoma progression to carcinoma and it was lower in tumors presenting myoepithelial differentiation, although showing high microvascularization total area. Cromossomic analysis has identified regions which genes potentially involved in tumoral process or the ones responsible for tumor severity.

MAIN PUBLICATIONS

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EVALUATION OF THE CLINICAL AND MOLECULAR REPERCUSSIONS OF THE USE OF CONTRACEPTIVES THAT CONTAIN ONLY PROGESTOGEN

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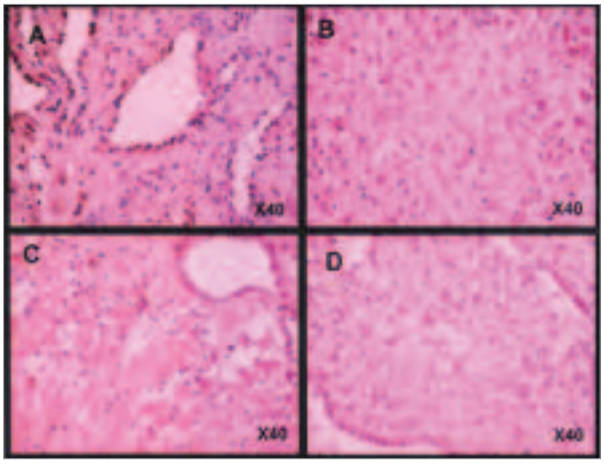

Immunohistochemical localization of chemokines in endometrium exposed to a levonorgestrel–releasing intrauterine system. (A) IL-8 in bleeding group, (B) IL-8 in non- bleeding group, (C) 6CKine in bleeding group, (D) 6CKine in non-bleeding group. IL, interleukin

There has been a trend towards the use of contraceptive methods containing just progestogen, among which implants, emergency contraception and SIU-LNG. With the increase in use, clinical evaluation has become important. This project aims to study aspects of contraceptive implants as endometrial alterations in women with and without bleeding, via metalloproteinase expression and microarray (cDNA). Also the prevalence of persistent ovarian follicles will be evaluated, an observation that is more frequent during the use of such methods. In addition, we will prospectirely evaluate the effect of the implants on bone mass. We have also studied emergency contraception (EC) with levonorgestrel, focusing on the acrosome reaction *in vitro*. We wish to study the calcium influx and the acrosome reaction *in vivo* in spermatozoids obtained from a uterine wash from women after the use of EC. Finally, the project also proposes new studies on the three monthly injectable medroxiprogesteron, byevaluating the function of the FSH isoforms during the use of this method.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Regarding the *in vitro* and *in vivo* influence of LNG upon sperm functions, we showed that the *in vitro* addition of LNG to capacitated human spermatozoa in amounts similar to those observed after the administration of LNG as EC did not affect the acrosomal reaction rate neither the calcium influx or tyrosine phosphorylation. However, when the quantity of LNG was higher and similar to that observed in users of the LNG-IUS, the *in vitro* LNG was able to affect the detection of D-mannose binding sites or pellucid zone (ZP) receptors. We evaluated whether the *in vivo* administration of LNG as EC to women was able to affect acrosomal reaction of sperm recovered from uterus and the expression of glycodelin-A in the endometrium. No effect was observed. These results changed the concept existing during the previous 30 years that the LNG as EC interfered with sperm functions.

In relation to the effects of the progestin-only contraceptives upon the endometrium we evaluated the effect in women with and without breakthrough bleeding (BTB) during use of progestin in order to understand the mechanism that provokes BTB in some women. The most important issue in the determination of BTB was mediated by inflammation. We are at present writing a manuscript about the endometrium of women with and without BTB in which we used the microarray technique. The results corroborated the inflammation as the main factor in BTB.

Regarding the clinical effects of the progestin-only contraceptives, we evaluated the effect on bone mineral density (BMD). We published the first study on BMD among users of the LNG-IUS which showed no deleterious effect when compared to controls. We are currently evaluating the same cohort at 10 years of use. Also, our group was the first to compared BMD in users of two models of subdermal contraceptive implants at baseline, 18 and 36 months of use and these women will be evaluated again at 60 months of use. Additionally, we evaluated the pharmacokinetics of depot medroxyprogesterone acetate (DMPA) among HIV-positive women users and non-users of triple antiretroviral therapy, and the results showed no effect. This observation reinforces the use of this highly effective contraceptive by young women in an unconcerned manner in countries where the prevalence of HIV infection is high. Also, we provide insight about the value of FSH among women on the menopausal transition who used DMPA and are in amenorrhea in order to determine menopausal status.

MAIN PUBLICATIONS

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CLINICAL AND MOLECULAR ANTIGENS AND ANTIBODIES RELATED TO BLOOD CELLS

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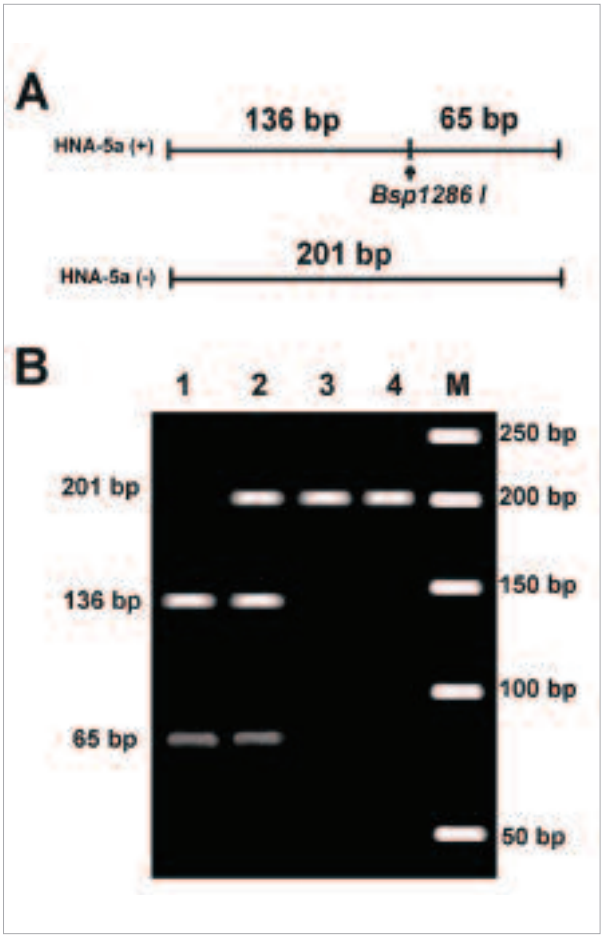
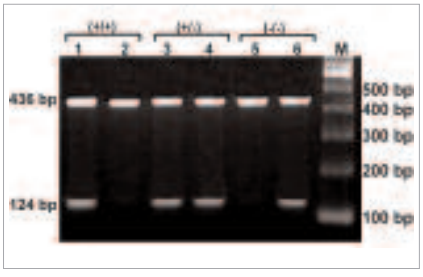


PCR-RFLP method and typical results of HNA-5a genotyping. A) A region (201 bp) in the genomic DNA, in which the HNA-5 polymorphism is located, was amplified by PCR. The sizes of the fragments produced by digestion with Bsp1286 I are shown. B) Typical RFLP patterns Bsp1286 I-treated PCR product. Lane 1: Homozygous HNA-5a (+/+). Lane 2: Heterozygous HNA-5a (+/-). Lane 3: Homozygous HNA-5a (-/-). Lane 4: not digested. Lane M shows the DNA molecular weight marker

Red cell alloantibodies may cause hemolytic disease of the newborn and hemolytic transfusion reactions, while red cell autoantibodies participate in the immune destruction of red cells seen in autoimmune hemolytic anemia. Neutrophil alloantibodies may produce neonatal alloimune neutropenia and transfusion related acute lung injury (TRALI), on the other hand granulocyte autoantibodies provoke neutrophil immune destruction observed in patients with autoimmune neutropenia. Platelet alloantibodies may induce neonatal alloimmune thrombocytopenia, post-transfusion purpura, and platelet transfusion refractoriness, conversely platelet autoantibodies induce primary or secondary immune thrombocytopenia purpura. The aim of the present immunohematological project is to examine not only the molecular basis of the blood cells alloantigen systems, but also calculate the risk associated to the exposition to allogenic blood cells by blood component transfusion, transplantation, or pregnancy.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The major project consists of 5 subprojects designed to study blood group systems: 1 subproject connected to platelet alloantigen systems; and 6 subprojects associated with granulocyte alloantigen systems. We propose to study immune mechanisms which take place in autoimmune hemolytic anemia, in homolysis post-kidney transplant, and in vaso-oclusive crisis seen in patients with sickle cell disease. We will also investigate molecular aspects of the Rh blood group system in Brazilian individuals, the gene frequency of some platelet alloantigen systems, and the platelet alloimmunization risk in Brazilian ethnic groups. Finally, we will determine the phenotypic and genotypic distribution of the granulocyte alloantigen systems in different Brazilian ethnic groups, the relevance of some polimorphisms in the expression of such antigens, and the role of granulocyte specific alloantibodies in some transfusion reactions.



Typical result HNA-4a genotyping by PCR-SSP. Lanes 1, 3 and 5 contain HNA-4a-positive-specific reaction, and lanes 2, 4 and 6 contain HNA-4a-negative-specific reaction. Lanes 1 and 2 contain HNA-4a homozygous-positive, lanes 3 and 4 heterozygous and lanes 5 and 6 homozygous-negative. Presence or absence of product at 124 bp determines genotype. All reactions also contained control HGH primers that resulted in a 432 bp product. Lane M shows the DNA molecular weight marker (from 100 bp)

MAIN PUBLICATIONS

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THE PROTOCOLS FOR THE POSTTRAUMATIC STRESS DISORDER PROJECT (PTSD): EPIDEMIOLOGY, PHYSIOPATHOLOGY AND TREATMENT

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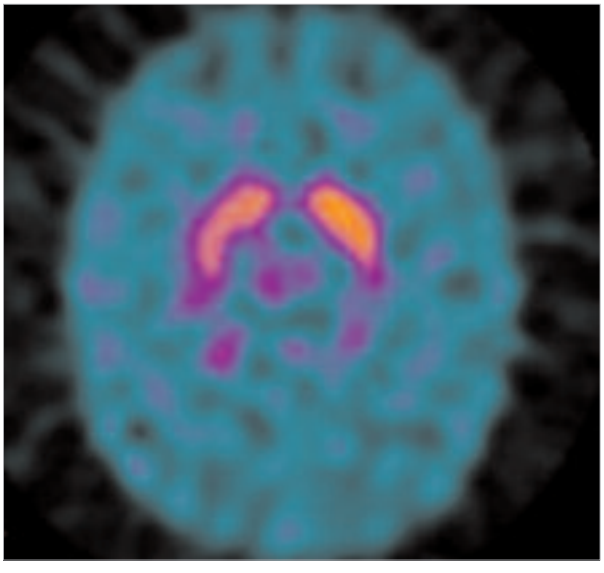

In vivo *cerebral dopamine transporter (DAT) scan with [99mTc]TRODAT-1 and SPECT of a healthy volunteer. The intense yellow reveals the density of DAT in the striatum. These technique will be used to evaluate the dopaminergic system of PTSD and reslient subjects*

To date no study estimating the prevalence of PTSD or any other mental health consequence of exposure to violence has been performed in Brazil. Well-designed epidemiological research can generate awareness, inform authorities responsible for policy, and encourage service development. The "Posttraumatic Stress Disorder Project (PTSD): epidemiology, physiopathology and treatment" refers to a research project which is being conducted in São Paulo City, Brazil, in order to study the impact of violence on the mental health of the Brazilian population. The study involves two phases, each focusing on different objectives and providing complementary information. The first phase is an epidemiological survey on exposure to violence among São Paulo City population and its consequences for mental health as well as a study for validating of the Brazilian version of "Clinician Administered PTSD Scale" (CAPS). The second phase comprises 6 studies on physiopathology of PTSD and two on treatment. The physiopathology issues includes: investigations on risk and protective factors for the disorder; genetic component on etiology; neuropsychological profiles associated with PTSD; the hypothalamus pituitary adrenal (HPA) axis activity in PTSD patients and its relation to traumatic event occurrence in childhood; and structural and molecular neuroimaging of patients and controls. The treatment of PTSD is approached in two studies: a systematic literature review on cognitive behavioral therapy for the treatment of PTSD, and a clinical trial examining the efficacy of topiramate. An animal model of PTSD has been developed to assess the neuroendocrinological and behavioral long-term consequences of maternal deprivation.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

- Cognitive behavioral therapy (CBT) is the most common psychotherapy approach for the treatment of PTSD. Our findings suggest that specific therapies, such as CBT, exposure therapy and cognitive therapy are equally effective, and more effective than supportive techniques in the treatment of PTSD.

- The experience of early adversity is an important risk factor for the development of posttraumatic stress disorder (PTSD) and/or major depressive disorder (MDD) during adulthood. Our findings are consistent with the hypothesis that the different forms of biological dysfunction found in patients suffering from PTSD and MDD might be related to the timing of the trauma onset.

- Posttraumatic stress disorder (PTSD), one of the possible consequences of sexual abuse of children and adolescents, may be found in about 40 to 50% of the cases. Treatment with CBT reduces PTSD symptoms in sexually abused children and adolescents, with no differences between therapy with only the victim or with the victim and a family member. No studies compared CBT and pharmacotherapy or the efficacy of combined treatments.

- A critical review of scientific literature showed that stress can be divided in stages to facilitate specific terminological adjustments to the event itself, to the subject-event interaction and to psychological responses. This study updates the etymological origins and applications of these words, connects them to the expansions of meanings that can be operated in the clinical care of patients with posttraumatic stress disorder, and analyzes them critically according to the criterion A of DSM-IV and ICD-10.

- Child maltreatment has been associated to different psychiatric disorders. The pathophysiology of posttraumatic stress disorder appears to be related to a complex interaction involving genetic and environmental factors. In contrast with studies involving adults, where the hippocampus volumetric reduction is the most consistent finding, studies involving children and adolescents with posttraumatic stress disorder have demonstrated smaller medial and posterior portions of the corpus callosum.

- A proposed explanation for memory impairments in posttraumatic stress disorder (PTSD) is stress-induced hippocampal damage due to elevated cortisol levels. Both reduced hippocampal volume and cognitive alterations have been consistently described in PTSD subjects. Our findings suggest that learning and executive functioning impairments observed in subjects with PTSD might be related to left hippocampal atrophy. Future follow-up studies examining larger samples are warranted to validate these preliminary findings.

- Early stress represents a major risk factor for psychiatric pathologies, including anxiety disorders and depression. Maternal deprivation (MD) for 24h during the stress hyporesponsive period may be a useful tool for the understanding of early trauma-induced neurobiological vulnerability to stress-related diseases. Our results indicate that MD induced a hyperesponsiveness to the psychological stressor.

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ANGIOTENSIN I – CONVERTING ENZYME ISOFORM (90 kDa), POTENTIAL GENETIC MARKER OF HYPERTENSION: PROCESSING, MOLECULAR AND FUNCTIONAL CARACTERIZATION, AND GENETIC SEGREGATION

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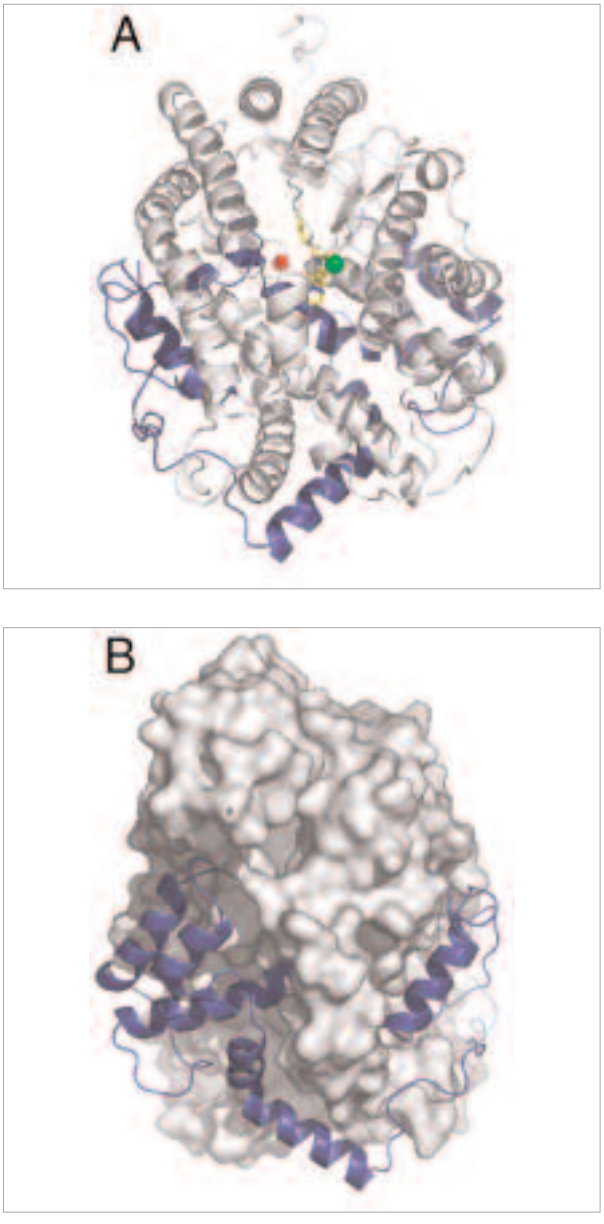

Cartoon and surface representations of the modeled 65 kDa protein, from (A) and rear (B) views. The region missing after truncation is depicted in blue. A, the inhibitor lisinopril modeled/placed in the active site is shown in yellow, zinc in green, and chloride in red

N-domain mRNA species have not been described in the literature. Thus, our objective will be to understand the mechanisms of formation and transport of ACE (Angiotensin Converting Enzyme) isoforms (190, 90/80 and 65 kDa), especially the 90kDa enzyme. The presence of N-domain ACEs in the cell nucleus could have an implication in the transcription to control new genes. The structural characterization and the sequence of the 90 and 65kDa N-domain ACEs of human urine and mesangial cells in culture will be done. The structure study of this marker can contribute to the development of new drugs for blood pressure control. In this project we will finish the standardization of the method for the quantification of ACE isoforms by western blotting (object of our patent).

Segregation of ACE isoforms will be done through a study of the genetic transmission of ACE isoforms – especially 90kDa ACE – in the crossing and backcrossing of SHR versus Brown Norway rats, in an 80-member family. It is our intention to use this marker to discover if its transference occurs after a renal transplant, just as the cells from the receptor start to get installed in the graft. In addition, the incidence of these isoforms will be evaluated in a determined population.

We will also study the association of ACE isoforms with renal and cardiovascular dysfunctions in humans and experimental models of acute and chronic renal failure, and diabetes in order to understand the physiopathology of hypertension. We will study the role of ACE isoforms in a pre-eclampsia model to assess the differentiation of gestational hypertensive disturbances, and in malnutrition related hypertension in infancy.

The aim of this project is of crucial importance in assessing ACE's role, and proposing their qualification methodology as a secure and useful diagnostic tool for predicting hypertension and target organ damage.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Membrane-bound angiotensin I-converting enzyme (ACE): secreted and intracellular forms of ACE have been identified in mesangial cells of Wistar and Spontaneously Hypertensive (SHR) rats by Andrade et al. (2006). N-domain ACE was present inside the nuclei of these mesangial cells. The structures of the 90 and 65 kDa ACEs were modeled. Ronchi et al (2005) described N-domain ACE expression in tissues of Wistar rats and SHR. The 90 kDa ACE had been previously described as a genetic marker of hypertension. The modulation of angiotensin peptides suggests that somatic and N-domain ACE, ACE2 and NEP could have important functions in the regulation of renal and pancreatic renin angiotensin systems through a counter-regulatory mechanism to protect the kidney in diabetes mellitus.

We evaluated the endothelial response in normotensive offspring with or without family history of hypertension and its association with the 90 kDa ACE in urine. In the presence of family history of hypertension and detection of 90 kDa ACE we noted a maximal flow mediated dilation of 12.1±5.0% versus 16.1±6.0% in those without previous history of hypertension and with no 90 kDa ACE, suggesting that the 90 kDa ACE may be related to the development of early endothelial changes. We studied the 90 kDa N-domain ACE prevalence in a population from Vitória/ES, Brazil. ACEs from 1150 subjects were identified by Western Blotting in their urine. 69.1% of the studied population presented in urine the 190, 90 and 65 kDa ACEs, 37.7% of whom were hypertensive. In 16.2% of the subjects, we detected in their urine the 90 and 65 kDa N-domain ACEs, all of whom were hypertensive. The 190 and 65 kDa ACEs were found in the urine of 14.7% of the normotensive subjects. The presence of the 90 kDa N-domain ACE in urine was therefore strongly associated with hypertension. These findings could contribute to the development of new efficient strategies to prevent and treat hypertension.

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NEUROSCIENCE IMAGING CENTER AT UNIVERSITY OF SÃO PAULO MEDICAL SCHOOL

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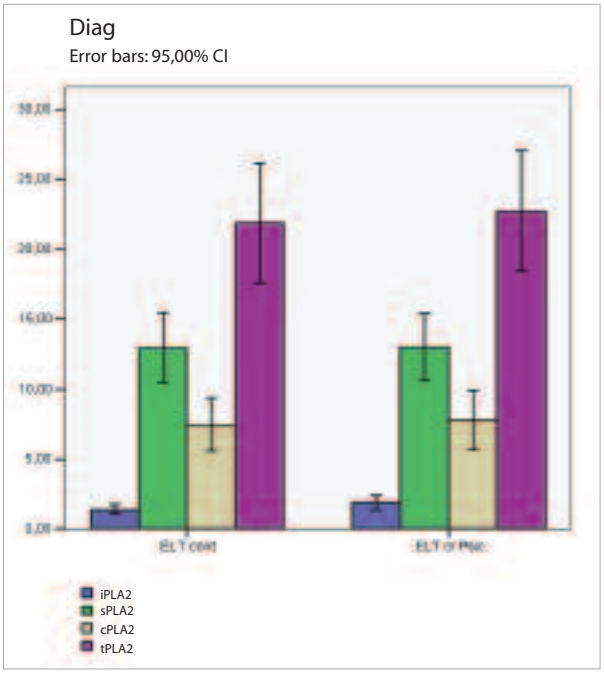

PLA2 activity in platelets is reduced in patients with mesial temporal sclerosis with psychosis without medication (ELT c/ Psic) compared to patients with mesial temporal sclerosis without psychotic symptoms (ELT cont). iPLA2: intracellular A2 phospholipase; sPLA2: secretory or extracellular A2 phospholipase ; cPLA2: cytosolic A2 phospholipase ;tPLA2: A2 phospholipase. All results are different (iPLA2: F= 3,092, df=6, p=0,008; sPLA2: F= 5,164, df=6, p<0,001; cPLA2: F=3,431, df=6, p=0,004; tPLA2: F=4,683, df=6, p<0,001)

Epilepsy is a complex disease in which abnormal cerebral activity is caused by increased neuronal excitability and decreased inhibition in large cerebral networks. Epilepsy can be either symptomatic (i.e. associated with a known or presumed structural brain lesion) or idiopathic (where genetic factors play a major role, and where seizures are present in an age dependent manner). Modern structural neuroimaging has allowed advances in the surgical treatment of epilepsy with improved identification of previously unrecognizable structural lesions, such as mesial temporal sclerosis and disorders of cortical development.

Recent advances in the field of genetics are starting to unravel the role of channelopathies in idiopathic epilepsies. Monogenic inheritance has been shown single gene defects in few cases of epilepsy. Polygenic inheritance is probably involved in most cases of epilepsy. Epilepsy should also be considered a complex neurobehavioral syndrome. Abnormal brain electrical activity has profound influences in cognition and behavior. Epileptic activity in the developing brain, in infantile and childhood epilepsies, may cause cognitive deterioration. In adults, cognitive dysfunction, such as language and memory problems, are known to occur in temporal lobe epilepsy associated with mesial temporal sclerosis, the commonest cause of medically refractory epilepsy in adults. Surgical treatment may aggravate cognitive deficits in temporal lobe epilepsy. On the other hand, neuropsychiatric symptoms may occur in close temporal association with seizures (ictally or peri-ictally) or as a more permanent phenomenon, in association with the disease itself (inter-ictally). The precise underlying mechanisms of such psychiatric symptoms are not yet established. The University of São Paulo Epilepsy-Neuroimaging Group proposes to carry out five research projects involving functional and structural neuroimaging aspects of cognitive and behavioral functioning in adult and pediatric epilepsy as well as aspects of genetics and neuronal interconnectivity. An integrated research team effort involving clinical neurophysiological, neuroimaging and basic sciences (genetics and mathematical models of neuronal connectivity) will allow for advances in the major fields of current epilepsy research. Below are the main highlights of this project recently started (2007).

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Brain function and plasticity are dramatically influenced by physical and chemical properties of the neuronal membrane. This membrane is formed by a double-layer of phospholipids, where receptors, ion channels and other proteins involved in signal transduction are immersed. The phospholipids also act as a substrate for the synthesis of inter and intra-cellular mediators, increasing their relevance in the neurotransmission process. Phospholipid metabolism is controlled by enzymes linked to the cellular membrane. In this process, phospholipase A2 (PLA2) is a key enzyme. Increased activity of this enzyme has been found both in patients with psychosis and epilepsy, resulting in accumulation of bioactive lipids in the cellular membrane. We have measured PLA2 activity in patients with mesial temporal epilepsy with and without psychotic symtoms.

The figure on page 1 illustrates these two main steps. We will proceed with investigating phospholipids *in vivo* by using phosphorus MRS.

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DIAGNOSTIC AND PROGNOSTIC MARKERS IN THRYOID TUMOR PATIENTS: TRANSLATION OF NEW DISCOVERIES INTO CLINICAL PRATICE

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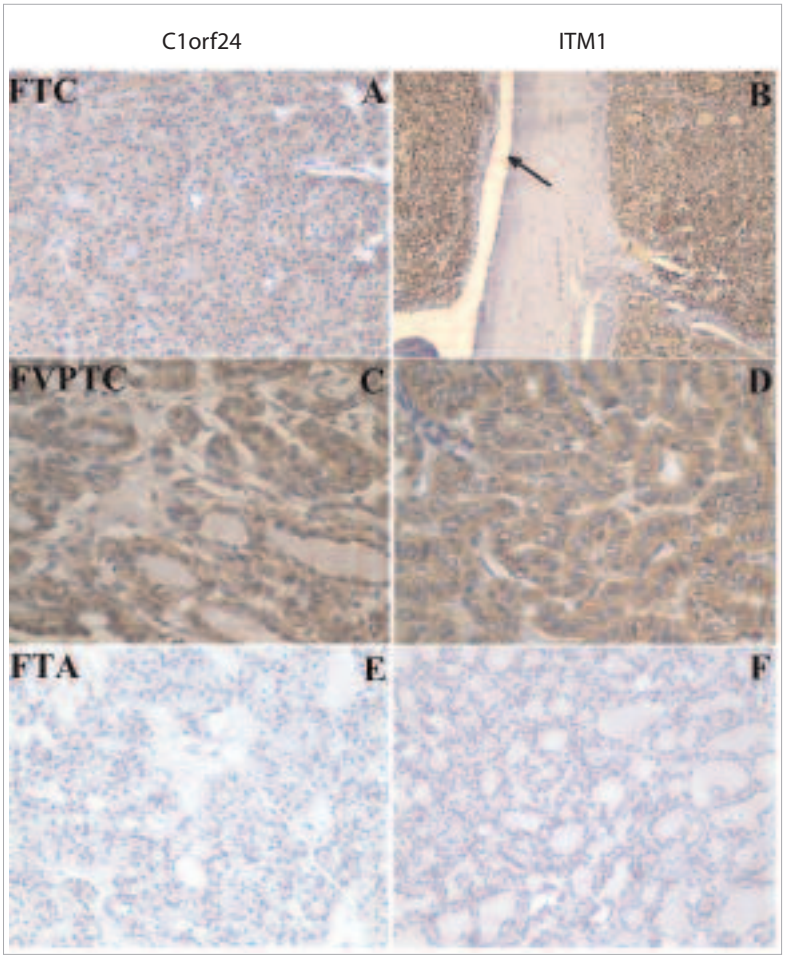
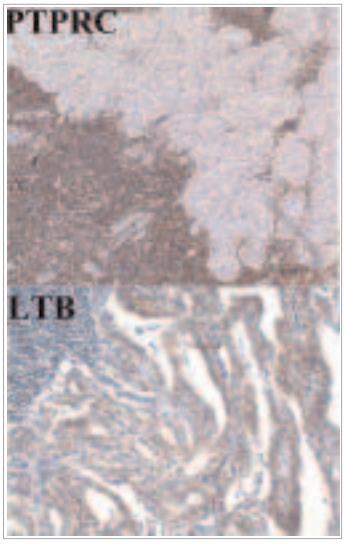


Immunohistochemical analysis of ITM1 and C1orf24 in paraffin-embedded sections of FTC (a,b), FVPTC (c,d) and FTA (e,f). Malignant tumors, FTCs, FVPTC, exhibited strong brown immunostaining for ITM1 and C1orf24. In contrast, the benign tumors FTA exhibited no immunoreactivity

Our long-term goal is to address two important clinical problems related to patients with thyroid tumors. The first one is the incidental discovery of impalpable thyroid nodules, in an estimated 20-67% of general population. Fine-needle aspiration (FNA) is considered the best initial diagnostic tool in the evaluation of thyroid nodules. However, inconclusive diagnosis (suspicious) occurs in nearly 5-40% of all FNA. Guidelines for evaluation and management of patients with thyroid nodules suggest that all patients with a cytological report of suspicious should be referred to surgery for a more accurate diagnosis. Only 5-20% of these suspicious nodules, when removed, are indeed malignant on histology. Molecular markers could help to avoid a large number of surgeries. We previously performed SAGE in a follicular thyroid adenoma and follicular thyroid carcinoma. This analysis and subsequent validation revealed that four novel markers (*Ddit*3, *Arg*2, *C1orf*24 and *Itm*1) differed in the two types of tumor. A linear combination of expression levels distinguished FTC from FTA with an estimated predictive accuracy of 0.83. In this project we propose to improve the sensitivity and specificity of our test and perform function analysis for *Itm*1 and *C1orf*24. The second clinical problem is that recurrence of thyroid tumors is high, ranging from 20-40% of patients with differentiated thyroid tumors. Lymph nodes account for 60-75% of all neck recurrences. This project proposes to identify genes that are involved in lymph node metastases. To this end, SAGE will be performed in matched normal thyroid, primary tumor and lymph node metastasis. We believe that, besides understanding the mechanism of metastases, the molecular markers identified from gene expression profile must offer an alternative in the follow-up of patients with thyroid carcinoma and ultimately may reveal a target gene for therapy.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Biomarkers of papillary thyroid carcinoma (PTC) metastasis can accurately identify metastatic cells and aggressive tumor behavior. Serial Analysis of Gene Expression (SAGE) was performed on matched-normal thyroid tissue, primary PTC and a PTC lymph node metastasis. The genome-wide expression analysis and further validation in a larger set of matched-samples identified *Limd2* and *Ptprc* as consistently different between the tumor and metastatic samples ($P < 0.0045$). *Ltb* had borderline significance. The PTC SAGE library was compared to the previous generated follicular thyroid carcinoma library (FTC) and normal thyroid, to identify papillary thyroid carcinoma (PTC)-associated transcripts. We identified three genes (*Cst6, Cxcl14, Dhrs3*) strongly associated with PTC. Additionally, *Cst6* and *Cxcl14* were positively correlated with the presence of metastasis and *brafV600E* mutational status. Noteworthy, *braf* mutation was previously associated with the presence of metastasis. Our findings suggested that these genes may be induced subsequently to *Braf* activation and, therefore, may be downstream in the *Braf/Mek/Erk* signaling pathway. To improve the accuracy of our test, custom antibody for C1ORF24 and ITM1 and commercially available DDIT3 and ARG2 were tested in 127 benign and malignant thyroid tissue sections (which are a source for diagnosis errors by immunohistochemistry – IHC). We improved this diagnostic test by adding C1ORF24 and ITM1 custom antibodies and demonstrating its use on a wider variety of thyroid pathologies. We recommend that testing of all four cancer biomarkers now be advanced to larger trials. Use of one or more of these antibodies should improve diagnostic accuracy of suspicious thyroid nodules from both tissue sections and FNA samples.



Representative results of immunohistochemical analysis. Strong rown staining for PTPRC was observed in the surrounding immune cells in metastatic lymph node. LTB was positive for tumor cells within a lymph node, as revealed by the brown immunostaining

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LEISHMANIOSIS IN BRAZIL: CLINICAL AND IMMUNOPATHOGENETICS ASPECTS OF THE HUMAM AND EXPERIMENTAL DISEASE

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The american cutaneous leishmaniasis (ACL) presents a large spectrum of manifestation, varying from asymptomatic and/or subclinical in resistant individuals to different forms in susceptible individuals, such as localized cutaneous leishmaniasis (LCL), borderline disseminated cutaneous leishmaniasis (BDCL), mucocutaneous leishmaniasis (MCL), mucosal leishmaniasis (ML) and anergic diffuse cutaneous leishmaniasis (ADCL), depending on the species and host immunoresponse (TCD4+ Th1/Th2).

In spite of the advances in the knowledge about the classic form of the disease, there are still many gaps regarding the parasite, *L. (L.) chagasi*, and the human immunogenetic system. The clinical spectrum of the human infection still needs more precise definition to improve the clinical diagnosis of infected, symptomatic and asymptomatic individuals, as well as suitable treatments for the different forms.

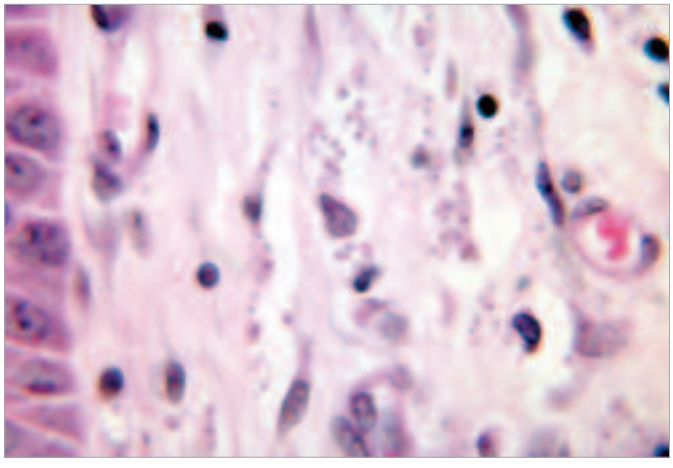
Longitudinal field studies are needed for identification of only infected, symptomatic and asymptomatic individuals. Long-term follow up for identification of the clinical immunological patterns of the human infection by *L. (L.) chagasi* is also needed.

The human immune response to infection by *L. (L.) chagasi* has received more attention in the classical form than in the symptomatic and/or subclinical forms of the disease, which have been neglected to some extent so far.

Experimental models for american visceral leishmaniasis (AVL) are usually made in hamsters and mice. However, data obtained from such models cannot be fully converted to figures comparable to those data that would expectedly be obtained in humans due to the philogenetic distances between the species, which makes a suitable experimental model closer to man highly desirable.

The *Cebus apella* has been shown as a potentially viable model for *in vitro* immune response studies using experimental infection in peritoneal macrophages for AVL and ACL species of *Leishmania* in Brazil.

The models of non-human primates, *in vivo* and *in vitro*, may be useful for immunopathogenical studies either for AVL or ACL.



Histopathology of leishmaniasis of skin due to Leishmania brasiliensis

The project aims at characterizing the role of the Langerhans cells in the CD4/CD8 immune response in the different clinical forms of the Brazilian ACL and different species in the immunopathogenesis modulation. An additional goal is the characterization of the clinical and immunological spectra of human infection by *Leishmania (L.) chagasi* in the Brazilian Amazon region, along with the humoral and cellular immune responses of the infected individuals.

Also proposed is the evaluation of the susceptibility of *Cebus apella* as an experimental model for experimental visceral leishmaniasis , *Leishmania (L.) chagasi*, and cutaneous leishmaniasis by *Leishmania (V.) braziliensis* and *L. (L.) amazonensis*.

In relation to the murine experimental model we proposed the kinetic analyses of the interaction between the Langerhans cells with the CD4+/CD8+ immune response in BALB/C e C57BL/6 mice elicited by *L. (V.) braziliensis* and *L. (L.) amazonensis* from different clinical isolates of ACL.

Mechanisms of resistance and susceptibility associated to the innate immune response of peritoneal macrophages from infected neotropical primates with *Leishmaia (V.) braziliensis*, *L. (L.) amazonensis* and *L. (L.) chagasi* will also be studied.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

A cross-sectional study on the clinical and immunological spectrum of human *Leishmania (L.) infantum chagasi* infection in the Brazilian Amazon region.

The objectives of this study were i) to identify individuals with symptomatic and/or asymptomatic infection due to *Leishmania (L.) infantum chagasi*; ii) to study the two types of infection, both clinically and immunologically, and iii) to determine the prevalence rate of infection at the beginning of the study. This was a cross-sectional study with a cohort of 946 individuals, of both sexes, from the age of one year on, living in an endemic area of american visceral leishmaniasis (AVL), municipality of Barcarena, Pará State, Brazil. For the diagnosis of infection, the delayed hypersensitivity skin reaction (LST) and the indirect fluorescent antibody test (IFAT) were used. One hundred and twenty cases of infection were diagnosed, with a prevalence rate of 12.6%; eight cases showed high seroreactivity (1.280 to 10.240, IgG) in IFAT and no reaction in LST; four cases being of typical AVL and another four cases of subclinical oligosymptomatic infection. The two immunological methods used simultaneously with the clinical examination enabled us to identify five clinical-immunological profiles: Asymptomatic Infection (AI) 73.4%; Subclinical Resistant Infection (SRI) 15%; Subclinical Oligosymptomatic Infection (SOI) 3%; Symptomatic Infection (= AVL) 3% and, Indeterminate Initial Infection (III) 5%.

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HEREDITARY HEMOGLOBIN DISORDERS: MOLECULAR GENETICS, CLINICAL FEATURES AND ANIMAL MODELS WITH THE PRODUCTION OF TRANSGENIC ANIMALS

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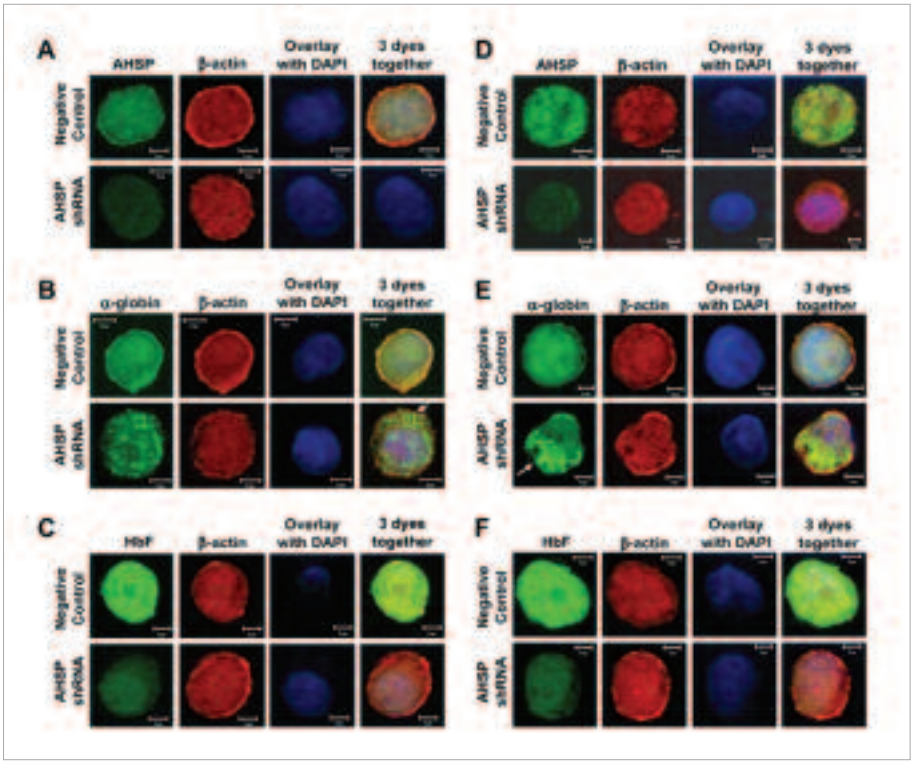

Immunofluorescence of hemin-induced K562 (A–C) and (D–F) represent immunofluorescence of hemin-induced K562 cells collected at 168 hours after hemin addition transfected by electroporation and Effectene, respectively. Bars: 5 µm. (A, D) Reduction of α-hemoglobin stabilizing protein (AHSP) production in AHSP–short-hairpin RNA (shRNA) cells (bottom and left) in relation to negative control cells (top and left). (B, E) α-Hemoglobin chain precipitation in AHSP-shRNA cells (bottom and left) in relation to negative control cells (top and left); the inclusion bodies are clearly identifiable (white arrows). (C, F) Reduction of fetal hemoglobin (HbF) production in AHSP-shRNA cells (bottom and left) in relation to negative control cells (top and left). Controls for immunofluorescence assays wereβ-actin–labeled cells and 4′-6′-Diamidino-2-phenylindole (DAPI) was used to stain the nuclear DNA

The Hemoglobinopathy Research Group of the Hemocentro and of the Department of Clinical Pathology, Unicamp, have, for nearly two decades, regularly investigated the hereditary hemoglobinopathies present in the Brazilian population. The environment of the State University of Campinas facilitates this type of study, since in addition to laboratory investigations, including the analysis of proteins and nucleic acids, the clinical evolution of patients is accompanied by the same group of clinicians and researchers, thus allowing for a large number of studies involving the association of both of these clinical and laboratory aspects.

The project consists of the study of gene expression in hematopoietic cells in hemoglobinopathies and hereditary anemias, the production of transgenic animals carrying genes that are important for the study of the hemoglobinopathies, and the analysis of the diverse pathophysiological and therapeutic aspects of the hemoglobinopathies, principally those involving vascular occlusion in sickle cell anemia and the mechanisms of action of nitric oxide.

Taken together, the project proposed herein focuses particularly on the study of the hereditary alterations of the hemoglobins. Despite the fact that it employs a large variety of methodological approaches along with clinical studies conducted in patients attended in four hospitals of the State of São Paulo, this project is expected to potentially produce important results for a better understanding of the pathophysiological mechanisms in the hemoglobinopathies. In addition, it is hoped to contribute to new therapeutic perspectives in these diseases.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The results of the project were extremely relevant for the achievement of a better understanding of the multiple mechanisms of action of hydroxyurea on the erythropoietic cells of humans. In addition, the studies regarding cell adhesion in sickle cell disease provide important results concerning the beneficial action of hydroxyurea and nitric oxide donors. Furthermore, data suggestive of the action of nitric oxide in the production of fetal hemoglobin were obtained.

The investigations of ASHP permitted the acquisition of original data regarding the importance of this protein in normal human erythropoiesis. Our findings on the actions of the GATA-1 factor in erythropoiesis, obtained by the study of a family that carried an extremely rare mutation, deserve particular emphasis. These data permit the formation of new hypotheses regarding the functions of the GATA-1 and GATA-1s proteins.

In fact, our group currently represents one of the most active groups in this area. As an example of this activity, we have published, in the last 3 years, approximately 42 articles in specialized journals with an international circulation, with various unedited contributions to the identification of mutations in structural hemoglobinopathies and thalessemias, molecular alterations of the blood groups in sickle cell disease, methods for the study of the flexibility of the red blood cells, identification of the genetic polymorphisms that modify the severity of sickle cell disease and the description of specific clinical aspects of the disease.

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PROSPECTIVE ANALYSIS OF VIROLOGICAL AND IMMUNOLOGICAL CHARACTERISTICS OF RECENTLY HIV-INFECTED MEN AND WOMEN IN SÃO PAULO AND SANTOS CITIES

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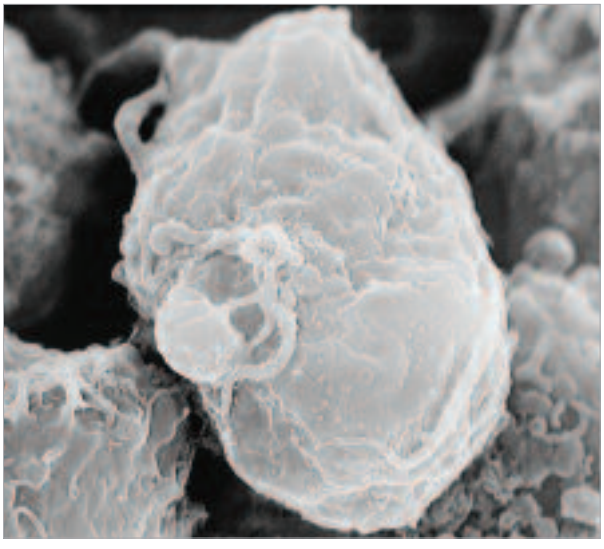

Scranning eletron micrograph of human imunodeficiency virus (HIV), grown in cultured lymphocytes. Virions are seen as small spheres on the surface of the cells

The objective of this project is to develop a well-characterized cohort of recently HIV-infected men and women and to carry out three prospective scientific studies in this group.

We are conducting molecular virology and clinical research investigating primary HIV resistant virus in the cohort and two hypotheses will be investigated:

Given the widespread availability of antiretroviral therapy in Brazil, we hypothesize that the prevalence of primary acquired drug-resistant HIV will increase over four years among newly infected persons We are ascertaining the clinical consequences of primary HIV infection with drug-resistant vs. wild-type HIV. We hypothesize that the rate of CD4+ T cell depletion will be slower in patients infected with drug-resistant HIV vs. drug-susceptible HIV, in part because the drug-resistant variant has reduced replicative capacity. However, the overall rate of disease progression will eventually prove to be greater in those with drug-resistant HIV due to reduced efficacy of antiretroviral therapy.

We are conducting immunological research aimed at understanding the breadth and cross-clade HIV-specific-cytotoxic T lymphocyte (CTL) responses directed against different HIV-1 gene-derived products. We will use state-of-the-art immunological technologies for clade-specific epitope mapping and investigate the role of the immune response in determining the virologic set point. We hypothesize that individuals with broader and stronger CTL response will have lower viral load set points, and we will investigate certain HLA alleles and their association with slower progression to immunodeficiency.

We are investigating and characterizing the unique epidemiology and natural history of the heterogeneous HIV epidemic that exists in Brazil, including assessments of: (i) the genetic diversity of HIV (the epidemic in Brazil is multiclade) and the potential for increased prevalence of recombinant viruses; (ii) the effects of widespread use of antiretroviral therapy and associations with risk in a developing country setting.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

By using the data from the cohort of recently HIV-1-infected subjects in São Paulo, and assessing the relationship between demographics, CD4+ and CD8+ T cell counts, HIV-1 RNA levels and presence of symptoms during acute HIV-1 infection on and over time prior to the initiation of anti-retroviral therapy, we have demonstrated an association between increased age and time to progression to immunodeficiency. Our results have strongly suggested that the presence of symptoms during acute HIV-1 infection does not predict progression to immunodeficiency. However, those patients with acute syndrome maintained higher CD8+ T cell counts and higher viral loads during their follow-up.

The full length HIV genomes generated from recently infected individuals, seeking attention at HIV counseling and testing sites in the city of São Paulo, showed that 83,3% were pure subtypes and 16,7% were recombinants.

Samples from individuals with primary resistance mutations were evaluated from 0 to 80 weeks of follow-up. We verified that mutations includes *tam* (20%), *M184V* (5%), *nnrti* (50%) and *pi* (35%). Only one individual lost mutation *M184V* after 12 weeks of follow-up. Strikingly, although viral load of recently infected individuals stabilized at a lower viral load level, as compared to average viral load observed among individuals infected with wild type strains, the CD4 decrease was more intense among individuals infected with wild type viruses.

HIV-1 is often acquired in the presence of pre-existing co-infections, such as Herpes Simplex Virus 2 (HSV-2). We examined the impact of HSV-2 status at the time of HIV-1 acquisition for its impact on subsequent clinical course, and total CD4+ T cell phenotypes. Among recently HIV-1 infected and sero-positive for HSV-2, there was no difference in initial CD8+ T cell count, or differences between the groups for age, gender, or race based on HSV-2 status. Persons with HIV-1/HSV-2 co-infection sustained higher CD4+ T cell counts over time than those with HIV-1 infection alone. We have also verified also that HSV-2 acquisition after HIV-1 acquisition had no impact on CD4+ count or viral load. We did not detect differences in CD4+ T cell activation or differentiation state by HSV-2+ status. We observed no effect of HSV-2 status on viral load. However, we did observe that treatment naïve, recently HIV-1 infected adults co-infected with HSV-2+ at the time of HIV-1 acquisition had higher CD4+ T cell counts over time. If verified in other cohorts, this result poses a striking paradox, and its public health implications are not immediately clear.

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PATHOGENESIS OF CARDIAC HYPERTROPHY AND FAILURE: MECHANISMS ACTIVATED BY MECHANICAL STRESS

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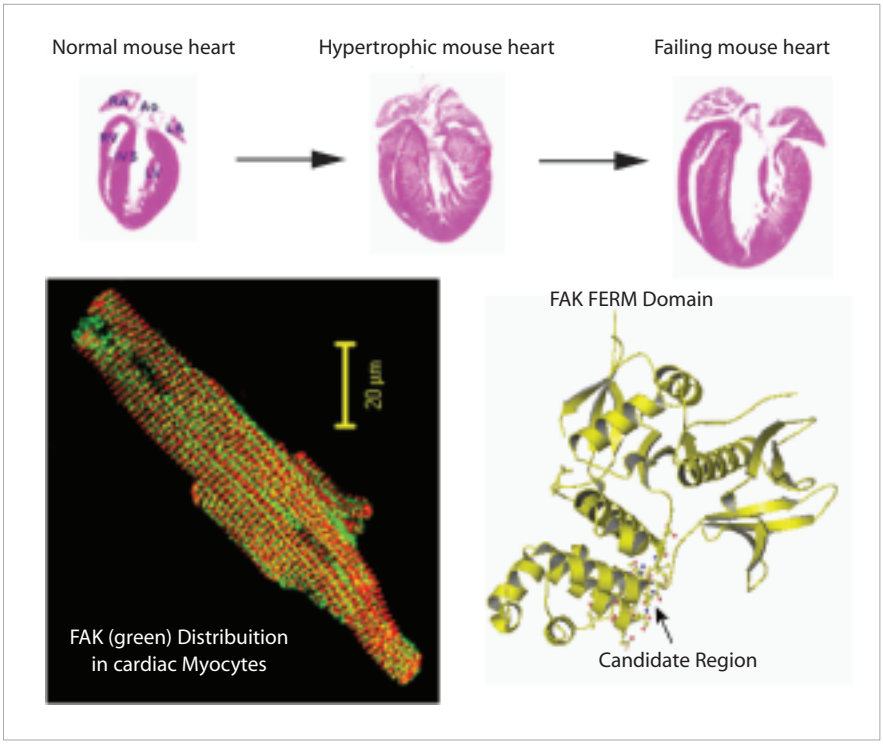

Example of structural changes in mouse left ventricle progressing from hypertrophy to failure. Example of adult mouse cardiac myocyte double stained with phalloidin (actin) and anti-FAK antibody. Structure of FAK FERM N-terminal domain indicating a mapped region explored for its role in the control of FAK activity.

Cardiac hypertrophy often accompanies cardiac diseases and is thought to set the stage for heart failure in the clinical settings of hypertension, myocardial infarction and valve diseases. Myocardial hypertrophy and its progression into heart failure are mostly driven by elevated mechanical stress, which leads to hypertrophy and injury of cardiac myocytes. The overall goal of our research plan is to identify the signaling mechanisms that control for the phenotypic and functional alterations of cardiac myocytes in response to mechanical stress. Our recent studies have been focused on the contribution of FAK (*focal adhesion kinase*) to the transduction of mechanical stimuli into biochemical signals, and the regulation of gene transcription during the hypertrophic growth of cardiac myocytes and the left ventricle. The research efforts are expanded to the development of synthetic organic compounds targeted at FAK.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

By using different models (i.e. cell culture, pressure overload induced hypertrophy, and failure in rodents and samples of human diseased hearts), we have found that FAK is activated in cardiac myocytes in conditions associated with mechanical stress. By using distinct experimental strategies that included overexpression of inactive mutant of FAK, pharmacological inhibitors and RNA interference technology, we showed that FAK is necessary for the mechanical stress-induced hypertrophy of cultured cardiac myocytes. More recently, by using a strategy of *in vivo* RNA interference, we confirmed the important role played by FAK in the pathogenesis of left ventricle hypertrophy in mice with chronic pressure overload induced by aortic constriction. Treatment with small interfering RNA targeted at FAK not only prevented the hypertrophy but also the progression of hypertrophic hearts to failure. These data indicate FAK as a potential target for the development of therapeutic tools aimed to control hypertrophy and prevent heart failure.

Also, we have used biochemical and structural biology approaches to identify the molecular mechanisms responsible for FAK activation by mechanical stress in cardiac myocytes. The experimental approaches include the mapping of regions potentially involved in intra and inter-molecular interactions, site mutations and the design of peptides to probe the importance displayed by specific regions of FAK in the process of its activation in cardiac myocytes. Another issue explored by our studies refers to the control of FAK activity by the tyrosine phosphatase SHP2. In addition, we have been using a two-hybrid system for screening a cardiac myocyte cDNA library and a combination of immunoprecipitation, protein separation by 2D-electrophoresis and identification of the resolved protein spots by mass spectrometry fingerprinting in order to identify partners that interact with FAK in non-stretched and stretched cardiac myocytes.

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PSYCHOLOGICAL STUDY OF ANTIDEPRESSIVE EFFECTS OF EMOTIONAL REGULATION

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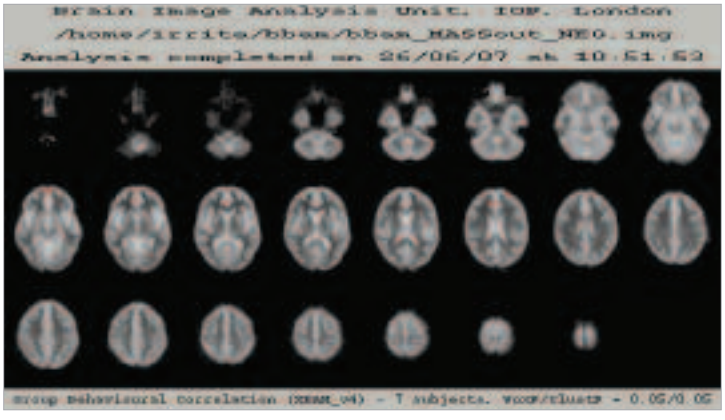
Antidepressants can induce positive changes in the subjective state of some individuals, independently of the presence of anxiety or depression. More specifically, decreased irritability, increased well-being, and a shift towards positive affect and a care-less attitude were reported in both clinical and normal subjects. The strategy of studying the effects of antidepressants in normal subjects is justified by the need to discriminate primary actions from therapeutic effects in clinical states.



Significant correlation foci between increases in BOLD signal and decreases in skin conductance levels in healthy, non-treated volunteers

The aim of the current project is to replicate and extend the scope of our previous investigations an the effects of low doses of antidepressants on the emotional response of normal subjects and patients with psychiatric disorders, in order to contribute to the understanding of the "extra-therapeutic" effects of this drugs and the mechanisms of mood regulation. The first phase the project involves clinical trials in normal volunteers without previous psychiatric diagnosis and studies with animals. The volunteers, grouped as asymptomatic or symptomatic, will receive flexible-doses of clomipramine, ranging from 10 to 50 mg or active placebo in a parallel group design, under doubleblind conditions, during six weeks, and will be evaluated before and at the end of treatment. The main objectives are: a) To assess the effects of antidepressants on personality characteristics, mood and other psychological variables, as well as, their relationship to cognitive processing, circadian rhythms, alimentary behavior and neuroimage; b) To explore the effects on the sleep-wakefulness cycle, evaluating the macro and microstructure of nocturnal sleep, and the objective and subjective parameters of daytime sleepiness, circadian temperature, activity and mood cycle, and effects on the secretion of melatonin; c) To study the effects of antidepressants on appetite and their relationship with weight gain and craving. Studies in rats will determine the effects of clornipramine on the ingestion of macronutrients, sucrose and sucralose, and saccarose craving; d) To explore the anatomic-functional correlates of emotional and motivational states before and after treatment with antidepressants. Measures of Functional magnetic resonance imaging will be obtained in different emotional conditions, before and after treatment with clomipramine. Depending on the results of these experiments, a series of clinical trials with antidepressants will be run in normal volunteers and psychiatric patients to test hypothesis regarding issues of specificity, validity and reproducibility of such effects. The improvement of the emotional responses of emotionally unstable individuals is a longstanding clinical goal with many favorable consequences for personal, family and social interactions. These studies may also help clarifying aspects of the limits of the pharmacological and psychological therapeutic interventions.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In the first double-blind, balanced, parallel design trial, individuals had received clomipramine or propanteline for 6 weeks. The extra therapeutic effect was identified as sustained changes in 3 out of 4 domains, as independently rated by 2 blinded psychiatrists. According to these criteria, 5 out of 24 individuals on clomipramine (21%), and 1 (5%) out of 21 individuals on placebo, were considered complete responders.

A second study confirmed the specificity of this observed response. After a single-blind clomipramine phase, volunteers, who reported the expected emotional changes, were included in a double-blind, placebo controlled phase. While on clomipramine treatment all individuals maintained (or e-acquired) response criteria.

These results had confirmed that, at low doses, clomipramine may induce qualitative changes in subjective state in the absence of psychopathology, in some, but not all individuals. Such perceived effects on mood, performance and social interaction, were consistently established and lost in a matter of days, and were not associated with sedation, psycho stimulation, or drug-induced hypomania, but represent a reduction in the expression of negative affect to aversive daily events. The mechanisms of action, individual response, and the clinical usefulness of this selective effect on mood regulation are still under investigation.

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SIGNALING EVENTS IN THE INTERACTION BETWEEN *Paracoccidioides brasiliensis* AND EPITHELIAL CELLS AND MONONUCLEAR CELLS INVOLVED IN THE IMMUNE RESPONSE

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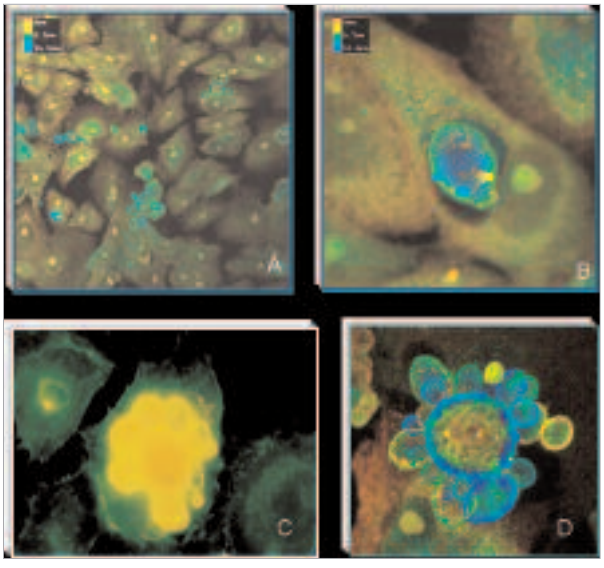

Adhesion and invasion process of P. brasiliensis *in Vero cells by confocal microscopy, after 2h (A) and 5h (B, C and D)*

Paracoccidioidomycosis (PCM) is an endemic deep mycosis caused by the dimorphic fungus *Paracoccidioides brasiliensis*. It is the leading endemic deep mycosis in Latin America, especially Brazil. The infection is acquired through the respiratory route. The conidia invade alveoli and terminal bronchi, where they transform into the yeast form. *P. brasiliensis* cause a mycosis with a wide range of clinical manifestations. Occasionally life-threatening depends on fungus virulence, its capacity to interact with the host, its invasion capacity and the immune response. With the purpose to evaluate the steps involved from the initial contact of *P. brasiliensis* with the host, the adhesion and invasion processes, our group developed interesting models of *in vitro* cell cultures. The interaction of *P. brasiliensis* with epithelial and mononuclear cells of human host seems to be determinant to the outcome of the infection and to the variability in the clinical presentation. Then, we propose to determine the genotypes of the fungus, to isolate and characterize the putative adhesins involved in this process, as well as the extra-cellular matrix components and to evaluate the fungal components that can modulate, through signaling events, the epithelial cell invading process. In parallel, we will study the participation of costimulatory molecules and the molecular aspects (Stats and caspases activation) related to PCM patients' vs. cured/ sensitized individuals lymphocyte proliferation and cytokine production after challenging with gp43, and to the apoptosis phenomena. We will also verify the role of costimulatory molecules in the induction/protection of/from apoptosis as a possible mechanism associated with the immunological imbalance of PCM. The simultaneous study fungy and host cells signaling and costimulatory pathways, will provide a better understanding of the mechanisms involved in the pathogenicity of *P. brasiliensis* and in the suppression of the Th-l response found in the patients, as compared to the healthy exposed individuals or cured patients. As a consequence, we will be able to conjunctly evaluate a higher range of cellular events associated with the pathogenesis of paracoccidioidomycosis.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Paracoccidioides brasiliensis possesses multiples factors that damage the host and contribute to its virulence phenotype. Aiming to understand the mechanisms that regulate the interaction of this fungy with host cells, we have studied the adherence, induction of cytoskeletal alterations, and differential signaling activity of the various surface molecules as well as the role of moleculy that may represent new microbial targets. Proteomic approaches allowed the characterization of fungal adhesins to extracellular matrix proteins. Adhesins expression was increased from yeasts freshly isolated from humans or after animal passage, displaying a higher adhesion capacity. Also, *P. brasiliensis* affects the cytoskeletal structure of the host cells and mechanism of invasion was shown to be microfilament and microtubule-dependent. In this study, we also show that the signaling pathways involved in the cell proliferation, growth, and cytosol signals associated with cytoskeleton were mediated by actin rearrangement, and the entry of the fungus into the epithelial cell apparently requires activation of the small family of RHO GTPases. An adaptin, a molecule that probably participates in the vesicular trafficking, and likely essential to yeast survival inside cells, was also described. In parallel, this study shows that the host-*P. brasiliensis* interactions leads to a distinct profile of costimulatory molecules expression in mononuclear cells from patients. This pattern is consistent with the hypoproliferative state ("anergic") of these cells, likely the main mechanism underlying the host failure in controlling the infection. Attempts to revert this anergy *in vitro,* by blocking the negative signaling of such molecules, were ineffective. We also detected an altered expression of STATS in patients with active disease, consistent with their biased, non-protective, TH-2 type cytokine secretion pattern. Most importantly, this pattern was modified by interfering on the local cytokine balance. The elucidation of the roles of fungal and host cells' immunoregulatory molecules may devise the development of vaccines and immunomodulatory interventions in this mycosis.

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MOLECULAR CHARACTERIZATION OF CONGENTIAL ENDOCRINE DISEASES THAT AFFECT GROWTH AND DEVELOPMENT

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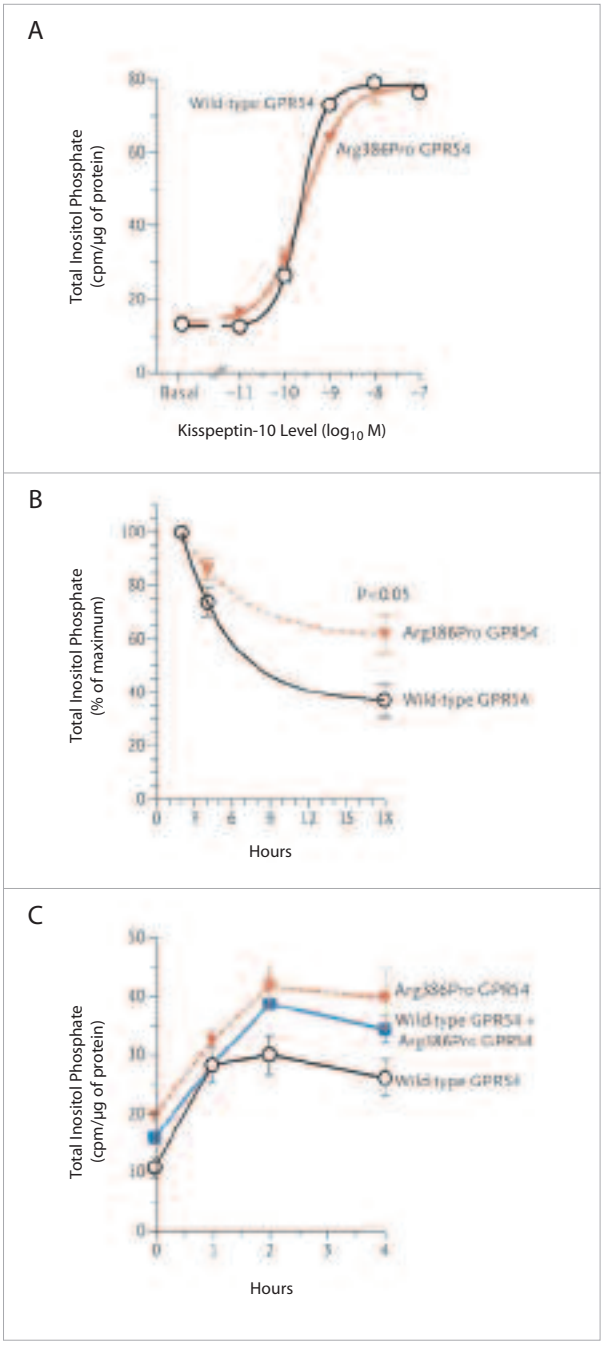


In vitro *study of wild type and mutated GPR54 in transfected cells*

Human growth is characterized by balanced cell multiplication, while development is characterized principally by cell differentiation. In this study, we propose to analyze the different genes in clinical syndromes related to human growth and development.

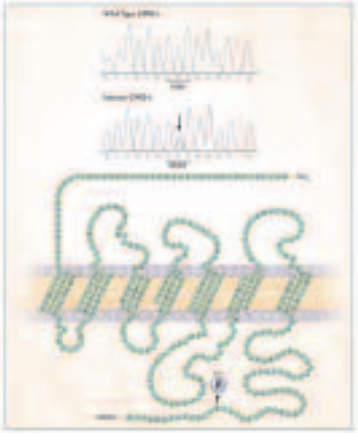
In the growth module we are analyzing different genetic defects implicated in the retardation of pre- and postnatal growth, most notably among them, the genes which codify IGF1 and its receptor (IGF1R), IGF2, insulin and the transcription factor PROP1. In addition to studying etiological factors of low stature, we will investigate the genic variations which can modulate the clinical response to treatment with GH.

In the development module, we are studying new genetic factors, recently implicated in gonadal determination (*Cbx*2, *Tcf*21, *Dhh* and *Foxl*2), in the hormonal regulation of pubertal development (*Fgfr*1, *Gpr*54, *Kisspeptin* 1) and in the etiology of hyperandrogenous syndromes (*Hsd*11β1, *H6pdh*, *Esr*1 and *Por*).

The identification and the functional study of molecular defects, in this group of patients, will contribute to the broadening of current physiopathological knowledge, as well as to the genetic counseling of these families. Finally, it may point the way to conditions that optimize the response to the treatment.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Several original and relevant results were achieved in the analysis of mutations or polymorphisms in genes implicated in human growth. Mutations in the *Ptpn*11 gene were detected in 39% of children of low stature carrying the Noonan syndrome. A polymorphism located in exon 3 of the receptor gene of the growth hormone (*Ghrd*3), involved in the therapeutic response to GH in children of low stature, positively influenced the sensitivity to GH established by the generation test of *Lgf*-1. In contrast to results previously published, allelic variants located in exon 6 of the *Lgf*1 gene were not associated with pre- and postnatal growth disturbances in Brazilian children.



Mutação Arg386Pro localizada na porção carboxiterminal do GPR54 associada ao fenótipo de puberdade precoce dependente de gonadotrofina

New genetic defects related to the morphogenesis and hormonal regulation of the secretory neurons of the gonadotropin-releasing hormone (GnRH), with consequent impact on secondary sexual development (*Kal*-1, *Fgfr*1, *Gpr*54, *Kiss*- 1 and *Prokr*2) were elucidated in patients with pubertal disturbances. New inactivating mutations in the *Kal*-1 and *Fgfr*1 genes were identified in patients with isolated hypogonadotropic hypogonadism. In a recent study, we have described a new mechanism for gonadotropin-dependent precocious puberty. We identified the first activating mutation in the gene which codifies GPR54, a receptor coupled to G protein with a fundamental role in the regulation of the secretion of GnRH. The ARG386PRO mutation in state of heterozygosis, located in the carboxiterminal region of the receptor, was identified in the genomic DNA of a girl with gonadotropin-dependent precocious puberty. *In vitro* studies demonstrated that this mutation results from the prolonged activation of the intracellular signaling in the presence of the kisspeptin ligand. This finding characterizes the first genetic cause of gonadotropin-dependent precocious puberty, thus opening a new field of investigation for the family cases of this type of sexual precocity.

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IDENTIFICATION AND CHARACTERIZATION OF ETIOLOGY, MECHANISMS OF DAMAGE, NEURONAL DYSFUNCTION, AND MOLECULAR DEFECTS IN MESIAL TEMPORAL LOBE EPILEPSY AND ITS RELATIONSHIP WITH RESPONSE TO TREATMENT

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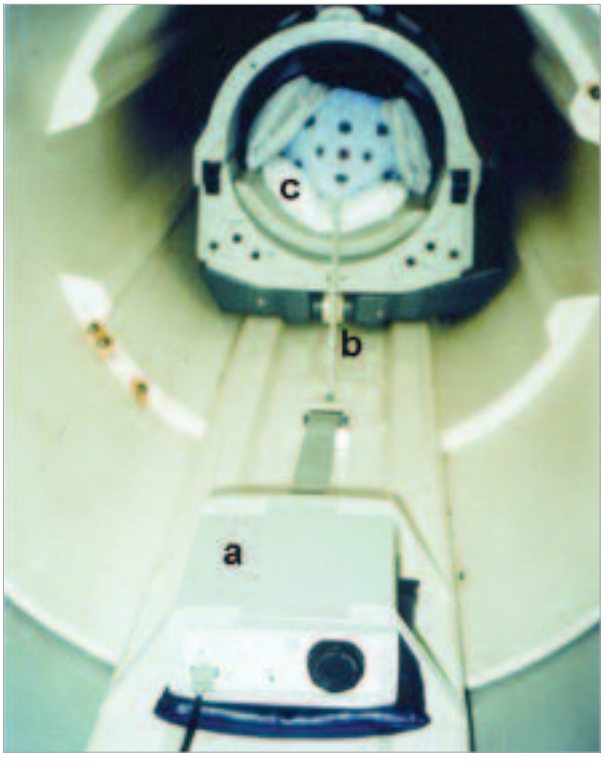
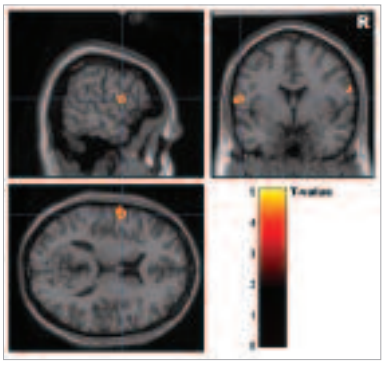


(a) fMRI Amplifier; (b) EEG electrodes and (c) BOLD

Epilepsy affects approximately 1-2% of the population and partial epilepsies with complex partial seizures account for approximately 40% of all epilepsies in adults. The most frequent form of partial epilepsy in adults is temporal lobe epilepsy (TLE). Classical histopathological studies and, more recently, different high-resolution neuroimaging modalities identify hippocampal atrophy and other signs of hippocampal sclerosis (HS) as the most prominent pathological substrate in patients with intractable mesial temporal lobe epilepsy (MTLE). Our aim is to perform a longitudinal study in a series of patients with MTLE and other forms of partial epilepsies. We will evaluate and quantify structural and functional brain abnormalities, by using different modalities of magnetic resonance (MR) imaging, and investigate the relationship between these abnormalities and the genetic substrate by using molecular genetic techniques. Specifically, we propose (a) to continue the development of MR techniques for evaluating TLE, including the use of functional MRI with simultaneous EEG recording (EEG-fMRI), (b) to study the relationship between the degree of hippocampal damage determined by MR techniques and anti-epileptic drug (AED) resistance in patients with MTLE, (c) to investigate factors related to prognosis for seizure control in patients submitted to surgical treatment for AED resistant MTLE; (d) to identify and characterize molecular genetic defects in patients with MTLE. We believe that this study will help to better understand the underlying pathogenic mechanisms in MTLE and other forms of partial epilepsies, and consequently, this may allow for a better diagnosis and treatment for these patients.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

One of the main achievements of the project was the construction of a database of normal controls including over 200 MRI scans of individuals of different ages. This database was used for comparison in prospective studies addressing the recovery of neuronal function accessed by different modalities of MR imaging and fMRI after epilepsy surgery. In addition, we have developed a number of tools for brain imaging segmentation that address specific biologic questions and problems such as description of structures based on different 'signatures' that can be defined by the user (i.e. shape and texture). These analyses are made based on the definition of patterns of asymmetry of each signature and the subsequent comparison between left and right brain hemispheres. These are based on forest-imaging techniques and implemented in a tool named BIA – Brain Image Analyzer. In the fMRI project we were able to establish relationships between patterns of activation in the brain and (i) the epileptogenic activity seen on the EEG of patients and (ii) the structural lesions detected by the different modalities of MRI. One of the major challenges of the project is the simultaneous acquisition of the fMRI signal and EEG recording which was successfully achieved by our group. The genetic studies of families with TLE lead us to conclude that a major locus segregating in an autosomal dominant pattern was involved in the predisposition to the disease. However, the same study also pointed out that minor effect genes are also modifying this effect, and are probably responsible for the clinical variability and differences in severity of the disease seen among patients. A genome wide search using 450 DNA markers was subsequently conducted and identified a major locus for familial MTLE on chromosome 18p in a candidate interval spanning 13 cM.



(c) BOLD response associated with EEG interictal activity in a patient with left temporal lobe epilepsy

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SEARCHING FOR MOLECULAR MARKERS RELATED TO DIAGNOSIS AND PROGNOSIS OF CENTRAL NERVOUS SYSTEM TUMORS

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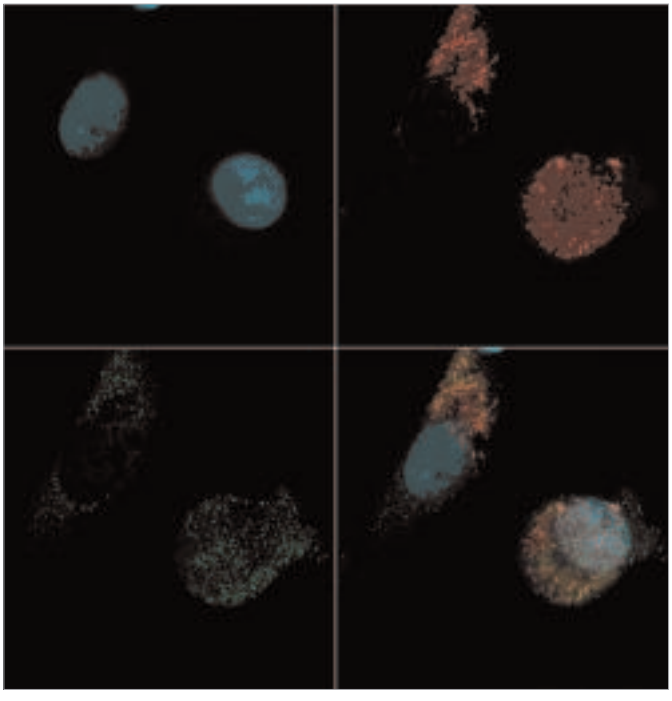
The main objective of the present project is to detect genes differentially expressed in central nervous system tumors to be useful as diagnostic markers, and as predictive factors of prognosis, in order to improve the therapeutical approaches, and to find future targets for gene therapy. As specific goals, this project was designed to pick up differentially expressed genes among astrocytomas of different grades of malignancy, medulloblastomas/PNET of child and adult, and oligodendrogliomas with and without LOH, by SAGE and microarray analyses.

Despite efforts to develop novel therapies, the median survival rate of patients with the most frequent and malignant brain tumor, glioblastoma, rarely exceeds 12 months, reflecting the resistance of these tumors not only to surgical approaches but also to chemotherapy and radiation therapy. Although multiple genetic alterations including chromosomal abnormalities, oncogene activation and tumor suppressor gene inactivation have already been identified in astrocytomas, the dismal prospects for patients with this disease render the identification of additional therapeutic targets as an important objective.

An oligonucleotide microarray study comparing pilocytic astrocytoma, a non-invasive grade I tumor with glioblastoma, a most malignant, and invasive grade IV tumor astrocytoma, disclosed very few genes differentially expressed. The gene expression of a set of those genes was validated by real time PCR, and its products were analyzed on tissue samples by immunohistochemistry. Polyclonal antibodies have been produced for the selected gene products for which there are no commercially available antibodies. Functional studies, including migration/invasion, proliferation, colony formation, and apoptosis assays, have been carried out *in vitro* and *in vivo* in immunossuppressed animals. Among the hyperpexpressed genes in glioblastoma are genes coding for surface membrane and extra cellular matrix proteins, which may be involved in the invasion progress of tumor. Also found were genes involved in cell proliferation and others with still unknown function. Genes on the aspect of tumoral invasibility and angiogenesis, on which a common mechanism of extracellular matrix degradation is observed, allowing the infiltration of these tumors, and genes particularly amenable for immunotherapy and for small molecule inhibitor therapeutical approaches have been considered as the major targets in our project. Additionally, the process of methylation of the promoter region was analyzed on hypoexpressed genes with unknown function. The question of radio and chemoresistency has been addressed on animal models as well.



KIAA0101 is one the genes selected as a potential therapeutic target. Confocal image showing the protein KIAA0101 (green), nucleus (blue) and mitochondria (red) in glioma cell line U87MG

Case-control studies of polymorphisms have been performed by using the samples of the DNA bank organized during the Clinical Cancer Genomics Project.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Sample collection obtained during the Genomics Project has been used for the studies, and new samples have been collected prospectively. Follow-up and treatment data have been continuously collected for all the patients included in the project. Over 240 tumoral samples and 100 non-neoplastic brain samples, besides the epidemiologic data, have been obtained until up to the present moment.

Different polymorphisms of different genes (*Egf*R, *Egf, Mmp*-9, *Ica*M-1, *Peca*M-1, *Itg*A2, *Tnf*A, *Tnf*B) involved in proliferation, invasion, adhesion and inflammation processes have been analyzed in case-control studies.

Genes upregulated in glioblastomas were selected from microarray analysis. Quantitative real time PCR and immnunohistochemistry have been used for the validation of the selected genes. To access the role of these genes in the tumors, *in vitro* and *in vivo* studies have been performed. Some genes were knocked out by siRNA technique. The role of *MelK* in tumorigenesis process has been demonstrated. Its expression is higher in the most malignant grades of astrocytomas. Functional studies of other genes, named *Kiaa*0101, *Dkfzp*762E1312, *Aspm*, *Plp*2, *Lox*, *Col*6A2 and *Hox*A5, are under analysis at the moment.

This Thematic Project has the participation of different research groups from different institutions: School of Medicine of USP from São Paulo, School of Medicine of USP from Ribeirão Preto, Federal University of São Paulo and Butantan Institute. There are other national multi-institutional cooperation with the Ludwig Institute for Cancer Research, São Paulo Branch, and Federal University of São Carlos. Additionally, international collaborative studies with the Ludwig Institute for Cancer Research, New York branch, and the Johns Hopkins University have allowed for technological transfers and results already published. The ongoing cooperation will refine the selection of genes as therapeutical targets, and speed up the development of therapeutical strategies for the benefit of our patients.

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MECHANISMS OF PULMONARY INFLAMMATION IN ASTHMA: CLINICAL AND EXPERIMENTAL STUDIES

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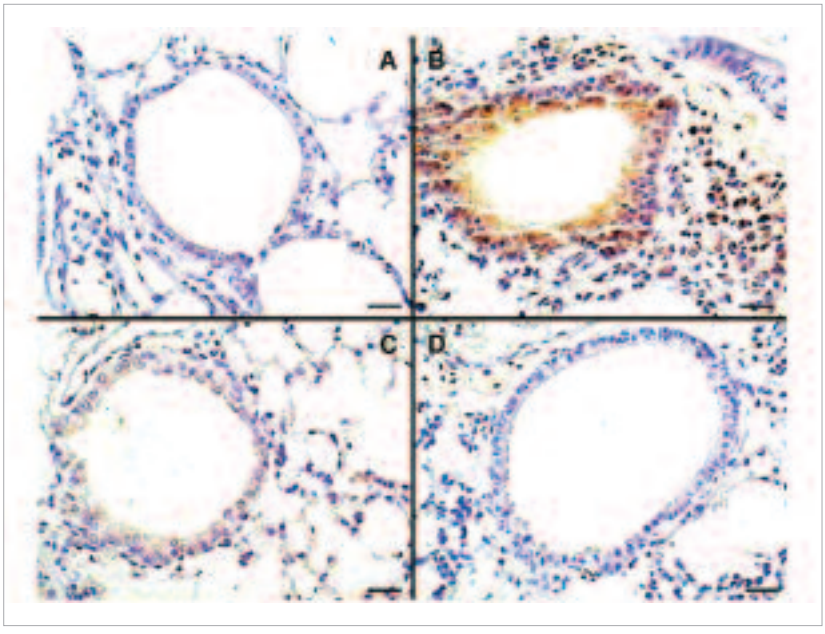
Asthma is the most common chronic disease in children and has also a high prevalence among all ages. The disease has a substantial economic impact and affects significantly the quality of life of asthmatic patients. It is well established that asthma is a specific type of chronic inflammation in the airways, although the precise etiology of this inflammation is not well understood. Multiple aspects of the inflammatory syndrome in asthma require more studies, including the precise role of each inflammatory cell type and the best way to evaluate the severity of the disease and the response to treatment. The understanding of asthma as a chronic inflammatory syndrome has influenced the development of multiple experimental and clinical studies concerning the pathophysiology of airway inflammation, the relationship between this inflammation and bronchial hyperresponsiveness, and the development of programs of long term care of asthmatics.



Effect of aerobic training on the expression of IL-4 by inflammatory cells in animal models of asthma. Photomicrographs of airways stained with anti-IL-4 in control (A), ovalbumin (OVA) (B) and OAV+aerobic trained mice (C and D), respectively. Note positive inflammatory cells in airway wall stained for IL-4

The central focus of our project was the pulmonary inflammation in asthma and we will perform studies with animal models of pulmonary allergic inflammation, studies with lungs and cell cultures from the lungs of people that died from asthma and also clinical studies with children and adults with asthma.

In this project we focus on:

1. The effects of nitric oxide and capsaicin-sensitive nerve fibers in the modulation of pulmonary inflammation in guinea pig and murine models of chronic airway inflammation.

2. The modulation of chronic airway inflammation by corticosteroids and leukotrienes using a guinea pig model of chronic airway inflammation.

3. The pulmonary expression and activity of nitric oxide synthase in experimental models of asthma and chronic obstructive pulmonary disease (COPD) and in people who died with asthma and COPD.

4. Evaluating the characteristics of airway inflammation in people who died from asthma or chronic obstructive pulmonary disease and correlation with clinical data.

5. The effects of physical training on airway inflammation, functional status and quality of life in children and adults with moderate and severe persistent asthma as well as its effect on airway inflammation in animal models of asthma.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our main findings were:

1) Aerobic training (AT) in asthmatic children reduced the severity of exercise-induced bronchoconstriction, post-exercise breathlessness and daily doses of inhaled steroids as well as improved health-related asthma quality of life scores. In asthmatic adults, AT increased the number of days without asthma symptoms and decreased the levels of eNO and eosinophil cells in the sputum.

2) In a murine model of chronic asthma induced by ovalbumin (OVA), AT decreased eosinophil counting in the bronchoalveolar lavage fluid and in the airway walls, and reduced the expression of IL-4 and IL-5 by peribronchial inflammatory cells. OVA-sensitized animals submitted to AT presented an increase the expression of IL-10 and a reduction in airway remodeling.

Conclusion: These findings suggest that AT is associated with beneficial effects on disease control and quality of life in asthmatic children and reduces airway allergic inflammation in asthmatic patients and in a murine model of asthma.

3) Airway inflammation in patients that have died due to asthma (fatal asthma) was investigated and we observed an increase in the number of eosinophils and mast cells in the outer area of larger airways, small membranous bronchioles and in peribronchiolar parenchyma. The number of CD3+, CD4+ and CD20+ cells was increased in the intrapulmonary airways. Increased neutrophil counting was also observed in peribronchiolar parenchyma.

Conclusion: Our findings provide further evidence of the importance of the lung periphery in the pathophysiology of fatal asthma.

4) The role of constitutive nitric oxide synthase (cNOS) and inducible NOS (iNOS) isoforms were investigated in a model of chronic allergic inflammation in guinea pigs. The inhibition of both NOS isoforms increased resistance of the respiratory system and collagen deposition on airways and decreased airway inflammation (edema and cell mononuclear cell migration). Specific inhibition of iNOS reduced resistance of the respiratory system, eosinophilic and mononuclear cell recruitment, and collagen and elastic fibers content in airways.

Conclusion: our findings suggest that both NOS isoforms modulates bronchoconstriction and airway inflammation and remodeling.

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INTEGRATED STUDY OF ISCHEMIC DILATED CARDIOPATHY: CHARACTERIZATION AND CONSOLIDATION OF ECHOCARDIOGRAPHY FOR EVALUATING CORONARY ANATOMY, AND MYOCARDIAL FUNCTION AND PERFUSION

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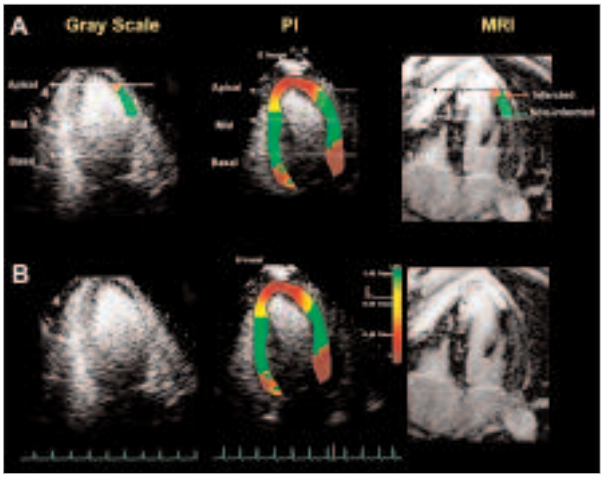

A. Myocardial contrast echocardiographic images showing how the measurement of infarct area (red) and myocardial segmental area (red + green) in the apical segment of lateral wall using Gray Scale, PI and MRI was performed. The infarct area was measured adding-up the infarct area in each infarcted myocardial segment and the total myocardial area by adding-up all 17-myocardial segmental area, in all modalities (Gray Scale, PI and MRI). B. End systolic frames from a patient with acute myocardial infarction. The infarct area in apical region is seen as a perfusion defect in Gray Scale, red color in PI, and hyperenhanced image in MRI. Gray Scale = measurement using low mechanical index gray scale; PI = parametric imaging, MRI = magnetic resonance imaging

Similarly to ischemic heart disease, dilated cardiomyophathy has a high worldwide incidence and prevalence, affecting over 1% of the world population, reaching more than 5% people over 65 years. Nowadays, the complete evaluation of the clinical data regarding coronary anatomy and functional repercussion of a given atherosclerotic lesion requires several investigative methods, frequently leading to conflicting results, high costs, and sometimes resulting in difficulties in selecting the proper treatment (clinical or surgical). In the last 30 years, the cardiovascular diagnostic methods as nuclear medicine, magnetic resonance and echocardiography turned to be fundamental tools for the diagnosis myocardial ischemia and viability. However, false results usually occurs in 20% of such studies, therefore opening the doors for new specific research in order to minimize the impact of additional costs originated from new studies or improprieties in the cardiovascular care. The present proposal for this integrated study, that includes several physiopathologic and anatomic data in patients with ischemic heart disease and dilated cardiomyophathy using modern echocardiograpic modalities (Resting 2D and 3Dimentional, and under pharmacologic stresses associated to the use of echocardiographic contrast agent) is based in a wide objective of consolodating its value among all imaging modalities. Therefore, an integrated, large scale study, designed towards the aim of demonstrating the value of each clinical and scientific information obtained through a single exam, at a reasonably low cost, is extremely desirable.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Three hundred and twenty six patients that underwent effective dobutamine and adenosine stress RTMPE (Real Time Myocardial Perfusion Echocardiography), were studied. Quantitative coronary angiography was performed within one month from stress test and has been analyzed in 53 patients. Accuracy of EKG, WM, qualitative MP, and quantitative MP for detecting CAD during dobutamine were 61%, 76%, 76%, and 80%, while during adenosine were 70%, 70%, 76%, and 80%, respectively.

Also, evaluating the ability of RTMPE in predicting cardiovascular events, by multivariate analysis, the only independent predictors of events were detection of WMA during dobutamine stress RTMCE (Relative Risk=4.08; p=0.043), and perfusion defect by qualitative analysis of adenosine RTMCE (Relative Risk =4,41; p = 0.036). When considering the combination of WMA and perfusion defect either by quantitative or qualitative analysis, dobutamine (Relative Risk =4,65; p = 0.031) and adenosine (Relative Risk =4,17; p = 0.041) stress RTMCE were independent predictors of events.

In another evaluation, we were challenged by the fact that quantitative myocardial perfusion echocardiography (MPE) with parametric imaging (PI), has been shown to accurately measure infarcted area in animals, but not in humans. We sought to validate MPE quantification of transmural extent and size of AMI using magnetic resonance imaging (MRI), as a gold standard. Twenty patients (12 men, 64±13 years) underwent MPE and MRI between the 2nd and 5th day post AMI. Infarct area and location, number of involved segments and transmural extent in each segment were determined by PI. Results were compared to late enhanced MRI. There was 99% agreement between both methods regarding the segmental location. The correlation between infarct area by MRI and PI was 0.87;p< 0.001. The correlation between transmural extent by MRI and PI was 0.89;p<0.001.

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OCULAR SURFACE RECONSTRUCTION: BASIC, CLINICAL AND SURGICAL ASPECTS

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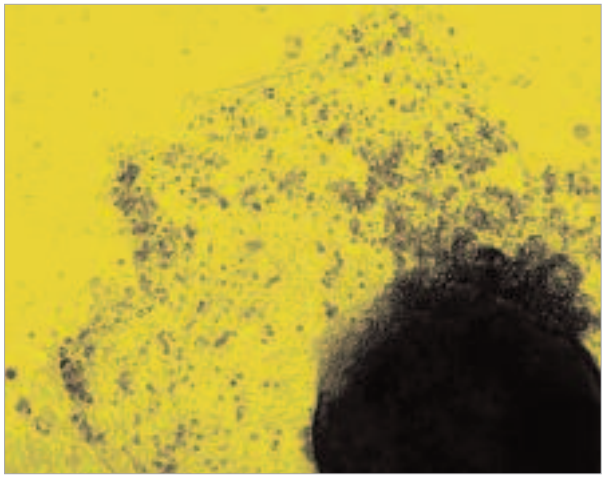

Epithelial expansion from a limbal explant cultivated ex vivo *(x40)*

Our study includes the following objectives: (i) to evaluate the ultra-structural, biochemical and molecular aspects of the amniotic membrane colonized *ex vivo* by corneal epithelium; (ii) to analyze the alterations of the ocular surface by different specialized diagnostic methods; (iii) to evaluate new treatments for dry eye; (iv) to evaluate the efficiency of reconstructive surgery of the ocular surface by using amniotic membrane associated or not with limbal transplant in Ocular Surface Disorder. Briefly we present the methods used for each protocol: scanning transmission electron microscopy in fresh amniotic membrane preserved by two different methods; quantification by ELISA – in both preserved and fresh amniotic membrane – of growth factors (recombinant human epithelial growth factor (EGF), fibroblastic growth factor (FGF-4, FGF-basic), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), beta-transformant growth factor (TGF-b)), interleukins (IL-4 e IL-10) and postglandins (PGE2); identification and quantification of growth factors and cytokines mRNA's in the human amniotic membranes by PCR techniques, "Northern blot" and "Western blot"; use of the amniotic membrane in experimental cicatricial keratoconjunctivitis in rabbits; ultra-structural analysis of cell proliferation (immunofluorescence with Ki-67), apoptosis (morphological method with Hoechst 33342) and immunohistochemical/FACS to determine the expression of the epithelial.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

This project includes the development of 15 research projects [5 basic research (PE), and 10 clinical research (PCC)]. The resources provided by FAPESP allowed us to install the Advanced Ocular Surface Laboratory (CASO), the first cell biology lab in Brazil focused on the development of cell therapy technology for ocular surface reconstruction.

Obtained results to date: Ultra-structure characterization, biochemical and molecular aspects of the human amniotic membrane. The evaluation of the efficacy of amniotic membrane and limbal stem cell transplantation for ocular surface reconstruction. Graft survival was observed with a cumulative survival of 33% after a mean follow-up time of 33 months. Increase in postoperative visual acuity was observed in 60.6% of the operated eyes during this period. Marked impact on graft survival was observed for patients with Stevens-Johnson syndrome, dry eye, keratinization, eyelid abnormalities, and allogeneic conjunctival limbal transplantation (independently of HLA compatibility) ($p<.05$). Preoperative dry eye was the most important prognostic parameter for surgical outcome ($p<.001$).

We are also completing three important prospective, comparative studies evaluating the efficacy of amniotic membrane in the treatment of pterygia, bullous keratopathy and corneal and scleral thinning.

We developed at the CASO the technique for amniotic membrane colonization with human limbal epithelial stem cells expanded *ex vivo*. In our hands, the best method includes the use of fragments of limbal tissue (and not cell suspension) over EDTA deepithelialized human amniotic membrane. We are also evaluating different cell lines (conjunctival epithelia and immature dental pulp stem cells) in order to compare the survival and possibility for clinical application.

We started to transplant these *ex vivo* limbal epithelial cultivated amniotic membranes in patients with total limbal stem cell deficiency with reasonable results in the short-term. More cases are now scheduled to be operated in order to obtain a better evaluation of the procedure and survival of the transplanted cells.

We developed the technique of impression cytology as a diagnostic tool for limbal stem cell deficiency and other diseases. We are now standardizing immunohistochemical analysis with different cell and proliferation markers.

We are in the process of evaluating, through a double blind controlled study, the efficacy of topical use of 0.05% cyclosporine A in the treatment of dry eye secondary to Sjögren`s syndrome.

Our thematic project is a multi-disciplinary project of the Federal University of Sao Paulo (UNIFESP) supported by FAPESP, with the participation of another two national and two international Universities (University of Nottingham, UK, and McGill University, Montreal, Canada).

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HIGH RESOLUTION STRUCTURAL MRI AND RECEPTOR IMAGING STUDIES IN REFRACTORY TEMPORAL LOBE EPILEPSY : *IN VIVO* AND *EX VIVO* ANALYSES

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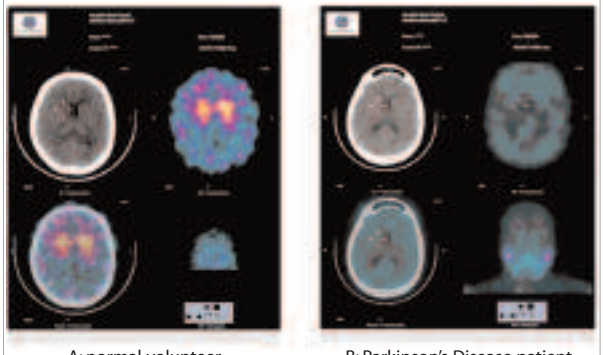
Molecular Neuroimaging: TRODAT-SPECT



A: normal volunteer B: Parkinson's Disease patient

Our main goal is the study of refractory mesial temporal lobe epilepsy (MTLE) by High Resolution Structural MRI, Receptor Imaging and historadiological correlations. This approach will allow the development of methods for correlating *in vivo* and *ex vivo* imaging. Accordingly, we devised three projects that will investigate the basis of MRI signal alteration, the profile of serotonin transporters detected by molecular imaging, and full analysis of brain parenchyma in patients with MTLE.

The resources (researchers, technicians, and imaging equipment) involved in the accomplishment of this proposal will make available to the CInAPCe network (Inter-institutional Cooperation to Support Brain Research): 1) a multidisciplinary team able to integrate clinical and experimental epileptology, innovative imaging acquisition/ processing and advanced histopathological analysis; 2) a state-of-the-art core facility for advanced imaging studies; 3) new methodologies for historadiological correlations; 4) new tools for second order imaging processing; 5) expertise on molecular imaging. These technological and scientific resources are applicable to other important areas of biomedical research (oncology, neurodegenerative and psychiatric diseases), and are designed to be integrated into a network environment sharing knowledge with other centers via e-learning. This is an essential requirement for the developing of telemedicine, a key tool for research, education and telediagnostic activities.

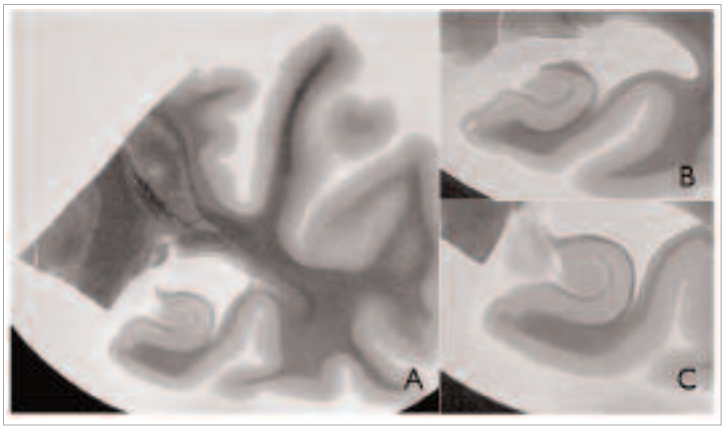
In spite of the importance of epilepsy to public health, the number of researchers and clinically qualified specialists in the related areas of neuroscience remains small. The CInAPCe network initiative is expected to promote a rapid progress in this field. Consequently, we are also responsible for disseminating the knowledge generated in the network projects in the medical and scientific communities. For this reason, the main educational emphasis of our program is on technology transfer of imaging processing methodologies by using voxel-based morphometry (VBM), molecular imaging acquisition techniques, and neuroimage databasing and data mining.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

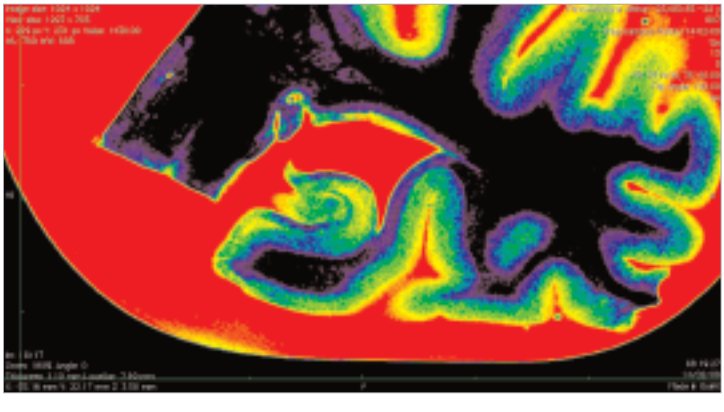
In the firsts months we conducted studies to determine the maximum spatial resolution achievable with our MR system. We have also calculated T2 relaxometry and contrast measurements in the hippocampal region. The figures below illustrate these two main steps. The results show an in plane resolution of 70 μm^2 which should be sufficient to our aims.

Related Research

The group is also involved in projects related to neuroimaging. The research is mainly related to Parkinson's Disease, neuro-oncology and cephalalgia. Molecular imaging of Dopamine transporters, functional Magnetic Resonance Imaging and diffusion tensor imaging are some examples (see figure on front page).



High resolution images of the hippocampal formation. A) Overview; B) and C) detailed information



Relaxometry of the hipocampal formation

MAIN PUBLICATIONS

Shih MC, Amaro-Jr. E, Andrade LAF, Felicio AC, Ferraz HB, Wagner J, Lin LF, Fu YK, Mari JJ, Tufik S, Bressan RA. 2007. Higher nigrostriatal dopamine neuron loss in early than late onset Parkinson´s disease? - A [99mTc]-TRODAT-1 SPECT study. *Movement Disorders*. **22**:863-866.

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PHYSICAL EXERCISE IN CARDIOVASCULAR PHYSIOPATHOLOGY

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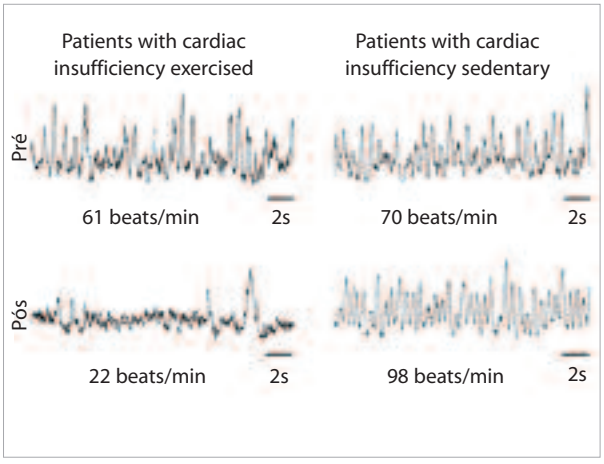

The aim of this thematic project is to investigate the effects of exercise on the cardiovascular physiopathology, which will be accomplished by studies in five subareas of the exercise cardiology and physiology. i) Heart failure and acute coronariopathy. Study of the acute and chronic effects of exercise in a genetic model of heart failure by sympathetic overactivity on the heart and in patients with chronic heart failure, in a clear association between animal and human researches. Our expertise in autonomic control will be also used to study the neurovascular control during the acute phase of the myocardium infarction in humans. ii) Metabolic syndrome. Study of the impact of exercise training associated with hypocaloric diet or hypocaloric diet isolated on the mechanisms underlying the sympathoexcitation in patients with metabolic syndrome. The effects of exercise training and hypocaloric diet on the myocardium calcium transport and, consequently, the ventricular function in obese rats will also be studied. iii) Effects of exercise training on autonomic control in individuals with polymorphism. In order to improve our understanding about genetic influence on the exercise-mediated cardiovascular adaptation, we will study the effects of acute and chronic exercise on cardiac hypertrophy and muscle vasodilatation in individuals with naturally occurring polymorphism in the angiotensin converting enzyme, angiotensinogen B2-adrenoceptors, and endothelial nitric oxide synthase. iv) Cardiac autonomic control in athletes and patients with neurocardiogenic syncope atrial fibrillation. In this area, we will investigate the autonomic adaptations provoked by chronic exercise during different periods of exercise training in athletes. In addition, we will study the effects of exercise training and postural training in patients with neurocardiogenic syncope. Finally, a new approach during electrophysiological study in the treatment of atrial fibrillation will be tested. Exercise training, anabolic steroids and cardiovascular system. In this area, we will study the effects of exercise training associated with anabolic steroids on the cardiac remodeling, coronary blood flow, and cardiac and systemic renin-angiotensin system in rats.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In the control of arterial hypertension by physical exercise studies, we observed that a single session of physical exercise provokes a reduction in arterial pressure in the post-exercise period in elderly hypertensive patients, which is explained by a decrease in the cardiac deficit as a consequence of improved left ventricle filling. Recently we described how the reduction in arterial pressure is due to the improvement in the arterial baroreflex control, which modulates the sympathetic nervous system.

In the study of the effects of physical exercise on the autonomic control of cardiac insufficiency, we described that the sympathetic nervous system limits the muscular endothelium-dependent vasodilator response in patients with cardiac insufficiency, given that the intra-arterial infusion of phentolamine associated with acetylcholine restores muscular vasodilation during the mental stress of these patients. More recently, we studied the effects of physical training in cardiac and skeletal myopathy provoked by cardiac insufficiency.

In the study where we evaluate the impact of hypercaloric diet and physical exercise in the neurovascular control in obesity, we verified that the vascular function is diminished in obese children (8-12 yo) and that diet associated with physical training, in contrast to diet in isolation, restores the vasodilatadory muscular response in these children.

In a recent study, our group described that individuals carrying polymorphism in the codon 27 of the β_2-adrenergic receptor present increased muscular vasodilatory response during exercise and mental stress when compared to individuals who do not carry this polymorphism. This is the first time that the importance of the genetic mutation of the β_2-adrenergic receptor which occurs naturally in the population has been shown. In this line of investigation, we also described how obese women with polymorphism in the α_{2B}-adrenergic receptor have alteration in the vagal and sympathetic function during the exercise of hand prehension when compared to women who do not have this polymorphism.

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DEVELOPMENT AND IMPLANTATION OF NEW METHODS FOR STUDYING THE ACCOMMODATION AND CONTRACTIBILITY OF THE STOMACH. APPLICATIONS IN INVESTIGATIONS OF PHYSIOLOGY AND PHYSIOPATHOLOGY OF THE MOTILITY

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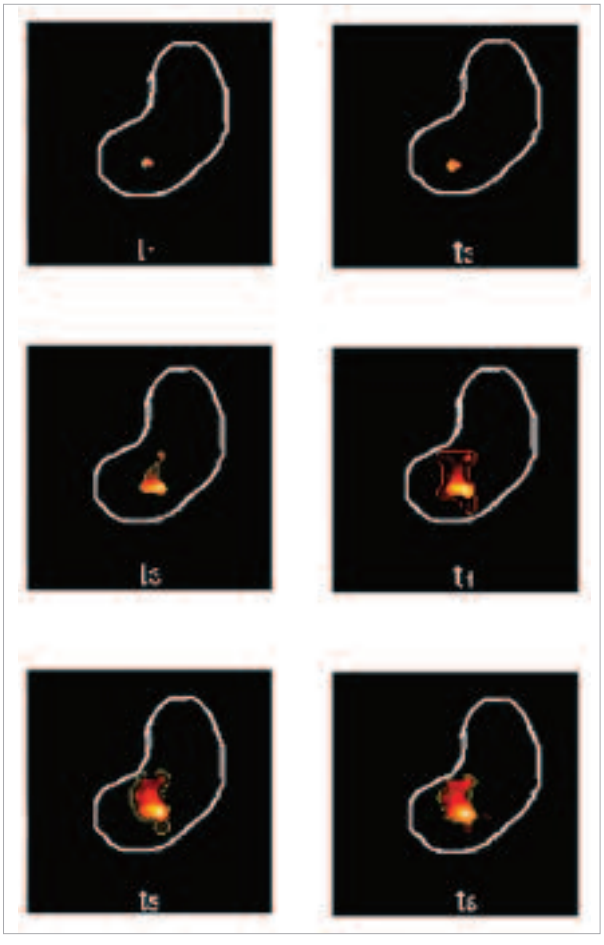


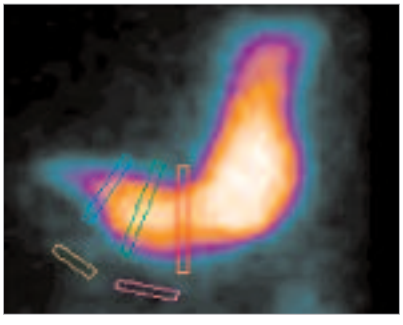
Magnetic images of the disintegration process of tablets in the human stomach by a biosusceptometry

The study of gastric motility has, for a long time, been carried out by means of invasive methods. More recently, non-invasive methods have been used, such as ultrasound, conventional scintilography, tomography by single photon emission and nuclear magnetic resonance. We are within a group active in the study of Digestive Motility, interest in the implantation of these methods, and the introduction of modifications which make them more efficient, as well as the development of other techniques, such as biomagnetic. It is planned, furthermore, to determine the effect of other variables of systemic nature, such as those related to volemic homeostasis, the relationships between the motor dysfunctions of the stomach, the control of appetite and the ingestion of foods, mediated by the liberation of neuropeptides, such as PYV, by endocrine cells existing in the distal part of the small intestine.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The project has provided vigorous support to the development and improvement of medical imaging techniques used for studies on gastrointestinal motility. Improvements of two previously described scintigraphic methods, for determining the time-course of gastric content distribution after meals, and for measurements of gastric contractility were made. The application of the improved techniques on patients with gastrointestinal motility disorders produced interesting results which were published.

A multidisciplinary team of researchers gathered within the project devised and developed a low cost, noninvasive biomagnetism-based imaging technique, which proved to be useful for a wide range of purposes: measurements of gastric emptying, intragastric meal distribution, contractility of the distal stomach, small bowel transit time, detection of "volume waves" in the proximal stomach , detection of disintegration of tablets in the gastrointestinal tract, and measurement of time-course of disintegration of tablets.



Scintigraphic image of stomach and regions of interest (ROIs) in the antrum

A couple of fruitful, medically-oriented lines of animal research, where experiments are performed on *in vivo* and *in vitro* models, are included in the project. The relaxing effects of Sildenafil (a vasodilator drug largely used for the treatment of male erectile dysfunction) on smooth muscle of rat stomach and duodenum was demonstrated. Involved mechanisms were described, the relationships of mucosal inflammations caused by NSAIDs and anti-cancer drugs, and their contemporaneous gastrointestinal motor dysfunctions have been clarified.

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FUNCTIONAL INVESTIGATION AND CHARACTERIZATION OF THE INVOLVEMENT OF NOVEL TARGET GENES AND NEW THERAPEUTICS FOR MYELODYSPLASTIC SYNDROMES AND LEUKEMIA LINEAGES

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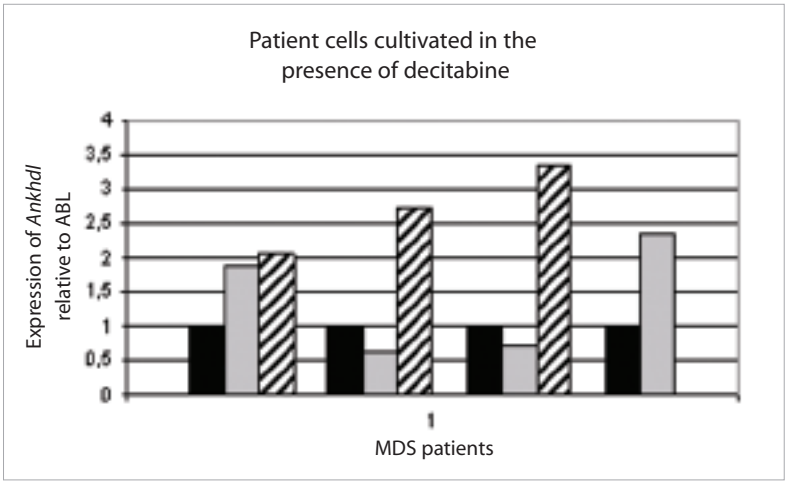

Myelodysplastic syndromes (MDS) are a group of heterogeneous hematopoietic disorders characterized by inefficient hematopoiesis. Little is known regarding MDS pathogenesis and the processes that mediate their frequent transformation into leukemia. During the last years it has become evident that composition and /or function alterations of the cellular microenvironment may be implicated in the progression of several hematological disorders, mainly MDS. New therapies have been proposed based on the biological characteristics of this type of tumor, however the molecular events responsible for the maintenance and dissemination of the anomalous clonal population remain unknown, frequently leading to the use of therapeutic agents that are not target specific. Therefore, the characterization of important molecular targets for differentiation processes and myeloid tumor progress could provide information, that would contribute for the creation of new specific drugs with greater and better action. During the Human Genome Project, several novel genes were identified, many of which presented great potential for therapeutic targets. This project proposes to characterize the regulation of novel gene expressions, specifically *Arhgap*10, *MasK*, and *Formin*, as well as other proteins, in myelodysplasias, submitted to different treatments, with the purpose of investigating molecular mechanisms of this type of tumor and the creation of new strategies for anti-tumoral therapy. Due to the nonexistence of cell or animal models with myelodysplasia, in order to fulfill some of the aims, we will use leukemia lineages as models. Furthermore mutations will be searched in genes that can associate evolving to leukemia such as *Ptpn*11, *Flt*3, *Aml*-1, *Gata*-1.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Apoptosis has a crucial role in myelodysplastic syndromes (MDS) and acute myeloid leukemia. Early disease MDS is associated with excessive apoptosis; apoptosis rate diminishes during disease progression. Cytochrome c/ APAF-1/ CASP-9 pathway is the main pathway involved in the apoptosis initiation by several stimuli. Original APAF-1 comprises of three functional domains; APAF-1XL and APAF-1LN isoforms have an insertion between CARD and ATPase domains and APAF-1XL has also an additional WDR. It was previously described that only the isoforms with the extra WDR activate pro-caspase 9. We hypothesized that APAF-1XL expression could be related to the higher rates of apoptosis found in early-stage disease of MDS and we showed , for the first time, that APAF-1XL is highly expressed in bone marrow cells of low risk myelodysplastic syndrome and in cells of patients with acute myeloid leukemia (AML) responsive to remission induction therapy. We are also interested in investigating the role of two new cytoskeletal genes (*Arhgp*21 and *Ankhd*1), previously described by our group in MDS and acute leukemia. Many interesting results were obtained: our data showed that *Arhgp*21 is overexpressed in AML and ALL (acute lymphoid leukemia) cells and is associated with FAK in leukemia cell lines and in normal peripheral blood. These findings raise the hypothesis that *Arhgp*21 may be involved in leukemogenesis, aiming this gene as a candidate for anti-tumor therapy. We also observed that *Arhgp*21 is upregulated by decitabine treatment in bone marrow mononuclear cells from patients with myelodysplatic syndromes and there is a positive correlation with □-catenin expression. Alfa-catenin deletion causes abrogation of cell differentiation and MDS. Ecitabine is an important agent for treatment of high risk MDS. Regarding the *Ankhd*1 gene, we observed that its expression is modulated by therapeutic agents in myeloid cell lines. The ankyrin repeat and KH domain containing 1 protein (ANKHD1) is protein homologue of Drosophila MASK (Multiple ankyrin repeats KH domain), which is known for its crucial role in photoreceptor differentiation, cell survival, and proliferation. We have demonstrated an upregulation of the splice variant *Ankhd*1 mRNAs expression during HL-60 and erythroblast differentiation. We showed that *Ankhd*1 is upregulated in KG1 and HL60 leukemia cell lines after treatment with α-Interferon or G-CSF in a dose-dependent way, suggesting its involvement in the regulation of differentiation and proliferation. Thalidomide is a drug used to treat low risk MDS. Our study also showed that thalidomide increases the number of CFU-GM and alters the cytokines expression profile in long-term bone marrow cultures from patients with myelodysplastic syndromes.

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SEPSIS: INTEGRATING BASIC RESEARCH AND CLINICAL INVESTIGATION

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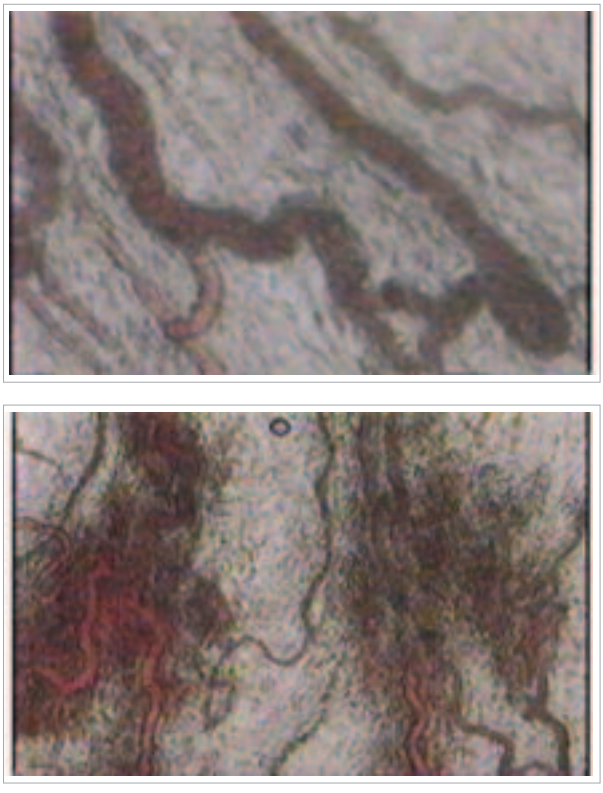

Mesenterial microcirculation two hours after bacterial translocation. Above, SHAM. And below, bacterial translocation

In spite of our best efforts, one out of two patients with severe sepsis and septic shock will still die. Thus, every one of us managing septic patients will be daily faced with the limits of our knowledge and intervention strategies, presenting us with a significant challenge for developing and improving patient care.

In this context, our research will be based on three interrelated lines of investigation:

Clinic and epidemiology.

The primary objective is to identify the main factors, both constitutive and related to therapeutic interventions, which are related to outcomes. The constitutive study will focus on genetic polymorphisms, whereas therapeutic interventions will focus on resuscitation, antimicrobial appropriateness, and support for organ dysfunction. The time elapsed to the correct intervention will be assessed to evaluate the time-dependent interventions. Samples obtained from these patients will be used for functional studies.

Translational Research – cellular response in patients with sepsis.

Cellular response to LPS is modulated during clinical sepsis, yet the mechanisms are only partially understood. The main objective is to evaluate the cellular response across the clinical continuum of sepsis. This includes cell surface expression, cytokine production, ROS generation and evaluation of the TLR pathway genes in PBMC and neutrophils in patients with sepsis, severe sepsis and septic shock.

Experimental models of sepsis.

Bacterial translocation is assumed to play an important role in severely ill patients with sepsis. The objectives are to evaluate the effects of bacterial translocation from gut in the modulation of inflammatory response in blood and in the lymph. The combination of systemic infection and bacterial translocation will be assessed regarding lethality, microcirculation alterations, and cellular functions in lymph and blood compartments.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our findings confirm the downregulation of inflammatory cytokines seen in severe sepsis and septic shock, and add evidence of an upregulation in earlier stages of sepsis, which may be related to pathogen recognition because it is more pronounced for LPS than for IL-1β, and TNF-α. In contrast, the downregulation observed in patients with severe sepsis and septic shock appears to be related to intracellular pathways common to LPS, IL-1β, and TNF-α.

Neutrophils and monocytes are activated in patients with sepsis, severe sepsis and septic shock considering oxidative metabolism. In the onset phase of sepsis, increased oxidative metabolism may be beneficial and is probably involved in resolution of the infectious course, but the persistence of high oxygen species formation in later stages may be associated with tissue damage and consequently organ dysfunction and death.

Our results show that the expression of TLR signaling pathway genes seems to be differently modulated in monocytes and neutrophils in patients with sepsis, severe sepsis and septic shock. Mononuclear cells presented downregulation in septic shock, predominantly in NFKB pathway, confirming the immunosuppressive nature of septic shock patients. On the other hand, neutrophils show predominantly upregulated genes, which comprise differential gene groups persistently upregulated across the stages of sepsis.

MAIN PUBLICATIONS

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POLYMORPHISM STUDY ON THE GENES RESPONSIBLE FOR THE BIOSYNTHESIS, ACTION AND METABOLISM OF SEXUAL STEROIDS IN ESTROGEN-DEPENDENT GYNECOLOGICAL AFFECTIONS AND DURING MENOPAUSE

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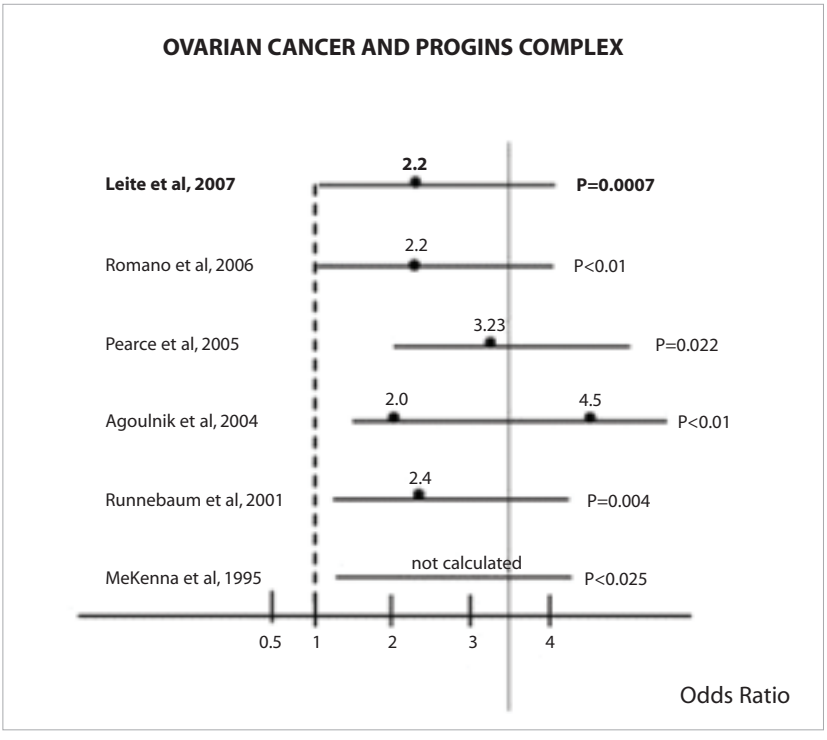
The present project is related to the study of polymorphisms located on genes involved in steroid biosynthesis, action and metabolism under estrogen-dependent conditions such as menopause, endometriosis, endometrial cancer, myoma, breast cancer and ovary cancer. The studied genes are *Cyp*1A, *Cyp*3A4, *Cyp*17, *Cyp*19, *Colia*1, *Comt*, *Gstm*1, Osteocalcina, Hepatic Lipase, Vitamin D Receptor, Androgen Receptor, Estrogen and Progesterone Receptors.



Comparative figure analyses among odds ratio values and P values obtained from different studies that found positive association between PROGINS and ovarian cancer

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

1- Menopause OBJECTIVE A: To investigate the influence of estrogen receptor-alpha (ESR1) gene polymorphisms on high-density lipoprotein (HDL) levels in response to postmenopausal hormone replacement therapy (HRT). CONCLUSION(S): ESR1 PvuIITT genotype is associated with increased levels of HDL after one year of HRT. OBJECTIVE B: To evaluate the presence of mutations in the coding region of the *Qm* gene and fragile X in patients with premature ovarian failure and gonadal dysgenesis. CONCLUSION: Our study suggests that *Xq*28 (*Qm* gene) may be involved in ovary failure.

2-Endometriosis OBJECTIVE A: To evaluate the association of intron 1 and exon 1 polymorphisms in the estrogen receptor alpha gene (ER-alpha) with endometriosis in women. CONCLUSION: The evaluated polymorphisms were not associated with endometriosis.

3- Endometrial cancer: The progesterone receptor gene (PROGINS) has been identified as a risk modifier for benign and malignant gynecological diseases. PROGINS polymorphism was examined in a total of 121 patients with endometrial cancer and 282 population-based control subjects, all located at the same area in São Paulo, SP, Brazil. The frequencies of PROGINS polymorphism T1/T1, T1/T2, and T2/T2 were 82.6%, 14.9%, and 2.5% in the endometrial cancer patients and 78.4%, 21.6%, and 0% in the controls, respectively. There was significant correlation between T1/T2 genotype and the presence of myoma (P= 0.048). No correlations were observed among the other variables. These data suggest that the PROGINS polymorphism T2/T2 genotype might be associated with an increased risk of endometrial cancer.

4- Ovary cancer A: PROGINS polymorphism was examined in a total of 80 patients with ovarian cancer and 282 control subjects. The frequencies of PROGINS polymorphism T1/T1, T1/T2, and T2/T2 were 71.3, 15.0 and 13.8% in ovarian cancer patients and 78.37, 21.63 and 0% in controls, respectively. These data suggest that the PROGINS polymorphism T2/T2 genotype might be associated with an increased risk of ovarian cancer.

5- Myoma OBJECTIVE: To assess the possible association between the polymorphic allele of the progesterone receptor gene, named PROGINS, and uterine leiomyomas. CONCLUSION: The PROGINS polymorphism revealed to be protective in terms of uterine fibroids in Brazilian non-white women.

6- Breast cancer Due to the conflicting results regarding the association between breast cancer and the GSTM1 null mutation, case-control study was performed on 105 women with breast cancer and 278 controls. The results conclusively show that single gene GSTM1 polymorphisms do not confer a substantial risk of breast cancer to its carriers. Furthermore, in this study no correlation was found between GSTs and smoking, reproductive history and several clinical pathologies with respect to cancer risk.

MAIN PUBLICATIONS

New approach reveals *Cd*28 and *Ifng* gene interaction in the susceptibility to cervical cancer. *Hum Mol Genet.* 2008. [Epub ahead of print] PMID: 18337305 [PubMed - as supplied by publisher].

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MECHANISMS OF HYPERTONIC SALINE SOLUTION ASSOCIATED TO PENTOXIFYLLINE AND ETHY-PYRUVATE IN THE REDUCTION OF MULTIPLE ORGAN DYSFUNCTION AFTER TRAUMA, SEPSIS AND ISCHEMIA-REPERFUSION

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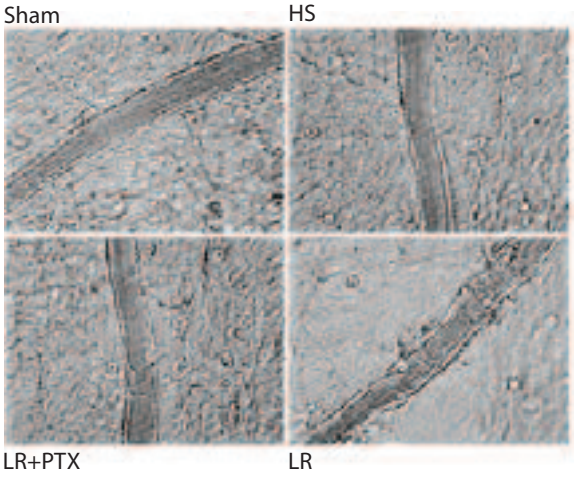


Representative image of intravitral microscopy of the spermatic fascia vasculature 2 hrs after shock and fluid resuscitation. No adherent cells were seen in the sham group. Lacted Ringer's (LR) plus pentoxifylline (Lr+PTX) and hypertonic saline (HS) treated animals had a very similar number of adherent polymorphonuclear leukocytes (PMN), which were markedly fewer than in LR-treated animals. (Yada-Langui et al. 2004)

Our goal is to investigate the mechanisms by which hypertonic saline solution, associated to pentoxifylline and ethyl-piruvate may attenuate multiple organ dysfunction after trauma, sepsis and ischemia-reperfusion, a major health worldwide problem due to its prevalence and to the high associated morbidity and mortality. We are testing the hypothesis that hypertonic saline solution, associated to pentoxifylline, ethyl-piruvate used in the treatment of hemorrhagic or septic shock, and ischemia-reperfusion of large territories induced by surgical maneuvers, decrease multiple organ dysfunction due to its beneficial effects would be particularly in the hepatosplanchnic area, through hemodynamic improvement at the macro and microcirculatory levels, by reducing ischemia-reperfusion injury and systemic inflammatory response, and preserving cellular function. We are employing clinically relevant experimental models of uncontrolled hemorrhagy, sepsis by live bacteria infection and peritonitis, and ischemia-reperfusion induced by surgical maneuvers, to evaluate the systemic and regional hemodynamic effects and the mechanisms by which such solutions may reduce multiple organ dysfunction in pigs and rats. Complex organ function monitored for a long period and the maintenance of these animals, in a facility similar to intensive care units, is a critical step before testing the potential clinical use of those solutions. In parallel, studies in small animal models of hemorrhage, sepsis and ischemia-reperfusion are being conducted, addressing important aspects of the microcirculatory, cellular and inflammatory effects of those solutions, which remains to be understood.

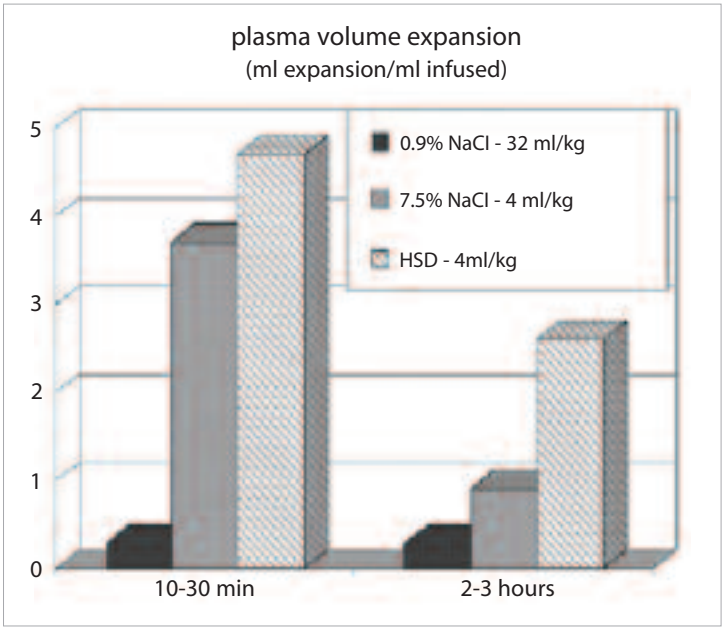
SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our results in large and small animal models of hemorrhagy, sepsis and ischemia-reperfusion are confirming our hypothesis that hypertonic saline and pentoxiffiline have potent synergistic beneficial effects, reducing organ damage, largely due to microcirculatory and cellular mechanisms.

Our treatment have reduced endothelial-leukocyte interaction, inflammation, organ dysfunction and apoptosis in uncontrolled hemorrhagy, sepsis due to live bacteria injection or cecal-ligation and puncture, and ischemia-reperfusion after aortic or mesenteric artery occlusion.

Our most striking observation is that all those microcirculatory and cellular benefits have been achieved in a small-volume resuscitation regimen with hypertonic-pentoxifilline, which promotes similar plasma expansion, and systemic and regional hemodynamic benefits as large volume crystalloids (8 times greater volume), the actual standard of care in the management of trauma victims, septic and surgical patients. The potential for clinical trials testing these hypothesis are real.

Plasma volume expansion in ml expansion per ml infused after 32 ml/kg isotonic NaCl, 4 ml/kg 7.5% NaCl, or 4 ml/kg 7.5% NaCl-6%Dextran-70



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MULTICENTER COOPERATIVE PHASE 3 STUDY FOR THE TREATMENT OF RECENTLY DIAGNOSED MULTIPLE MYELOMA: A RISK BASED STRATEGY

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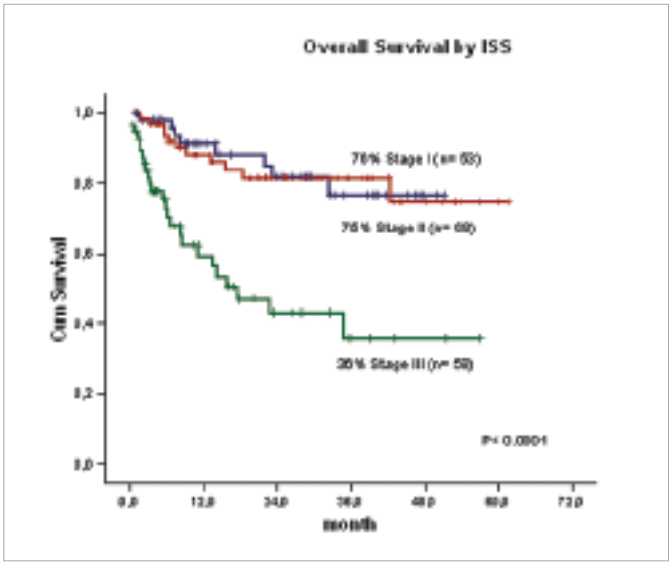
Our main objectives are:

To validate stratification by risk, proposing therapeutic strategies that vary in intensity, to evaluate the role of thalidomide in association with dexamethasone (low risk) or DCEP (high risk) as a consolidation scheme after the autologous transplant. This is a prospective, comparative multicentric, randomized and open study with two treatment groups, in accordance with risk stratification. Patients who do not show cytogenetic alterations involving chromosome 13 (analyzed by the FISH technique and conventional cytogenetics) and/or have a beta-2-microglobulin dose of 2.5 mg/l or less will be considered low risk patients, while high risk will be defined as those showing the presence of both alterations above. Treatment for the low risk group will consist of three chemotherapy cycles with the VAD scheme (vincristine, doxorubicin, and dexamethasone) administered at the outpatient clinic, followed by mobilizing the hematopoietic stem cells of the peripheral blood with cyclophosphamide (4g/m^2) and G-CSF (10 g/kg/day). After collecting a minimum of 4 x 10^8 cells CD34+/Kg of weight, the patient will be submitted to an autologous transplant, whose conditioning regime will be 200 mg/m^2 of melphalan. After D+100 of the transplant, patients will be randomized into two consolidation groups: thalidomide (200 mg/d) + dexamethasone (40 mg/d for 4 days once a month), in a total of 12 months, or dexamethasone (40 mg/d for 4 days, per month), in a total of 12 months. If there is relapse or progression of the disease, the patients will receive a second autologous transplant. Those who do not have enough cells collected (previous collection or new mobilization) will receive three cycles of monthly chemotherapy with the DCEP scheme (dexamethasone 40 mg/day for 4 days, cyclophosphamide 400 mg/m^2 for 4 days, cisplatin 10 mg/m^2 for 4 days and etoposide 40 mg/m^2 for 4 days) with or without thalidomide (200 mg/d), depending on whether or not they have used it before. For the high risk group, treatment will differ after the autologous transplant. Patients under 60 and identical HLA donors will receive a non-myeloablative allogenic transplant, and their conditioning regime will be the melphalan scheme (70 mg/m^2 /day for 2 days) and fludarabine (30 mg/m^2 /day for 4 days). As consolidation, these patients will receive infusions of lymphocytes from the donor on days D+60, 90, and 120 if they do not present acute GvHD. For patients above 60 or without compatible HLA donor, a second autologous transplant will be offered with the same conditioning scheme as the first. After D+100 of transplant, patients treated with the second autologous transplant will be randomized to receive chemotherapy with DCEP every three months, during a year (totaling four cycles) in one arm, and DCEP with thalidomide 200 mg/d for a year in the other consolidation arm. This study will last five years and shall include no less than 71 low risk patients, in each maintenance arm. Response rates, global survival and survival free from the disease will be analyzed in both groups, just as patients quality of life will be evaluated in the various phases of the protocol.



SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

ISS was highly predictive of prognosis in a Brazilian study in myeloma patients.

In 2003 the International Myeloma Foundation (IMF) group proposed a new International Prognostic Index (ISS). MM Brazilian group recently published in a retrospective analysis the utility of ISS in Brazil. This study presents the use of ISS in a multicentric prospective clinical trial performed in Brazil and its impact on survival. From October 2003 to January 2008, 229 untreated patients under 71 years old were enrolled in a prospective study developed in Brazil. All patients signed the informed consent and the protocol was approved by the Ethical committees. 190 patients were analyzed. 11 did not present data for ISS classification. At the end, 179 patients were included in this analysis, 53 (29.6%) ISS I, 68 (38%) ISS II and 58 (32.4%) ISS III; 93 (51.9%) were male and 86 (48.1%) female; the median age was 55 y (27 – 70) for the whole group; 38 (21.2%) Durie-Salmon II and 141 (78.8%) DS III. DS I were excluded from this clinical protocol. 132 out of 179 (77.6%) had 13q deletion analysis. For all patients, hematological (including BM), biochemistry and radiological tests/analyses were performed. The treatment was based on three phases: debulking with 3-6 VAD followed by high dose cyclophosphamide (4g/m^2) for mobilization plus ASCT and consolidation using dexamethasone with or without thalidomide. The statistical analysis was made by Chi-Square, Kaplan-Meier curves (log-rank test) and ANOVA. ISS I distributed by DS system showed DS II 15/53 (28%) and DS III 38/53 (72%); ISS II had DS II 15/53 (28%) and DSIII 38/53 (72%); ISSII had DSII 19/68 (28%) and DSIII 49/68 (72%) and ISSIII had DSII 4/57 (7%) and DS III 53/57 (93%) ($p< 0.0001$). Concerning the presence of 13q deletion, 12/36 (33%) ISS I, 16/51 (31%) ISS II and 17/45 (37%) ISS III (NS). The median observation time for whole group was 22 months and for alive patients 24 mo (1-62). 44 out of 179 (24%) died, most of them in VAD phase due to progression. 135/179 (76%) are alive, ISS I 45/53 (85%), ISS II 57/68 (84%) and ISS III 33/58 (57%) ($p< 0.001$). The OS in 60 mo by ISS was 76%, 75% and 36% for ISS I, II and III, respectively ($p< 0.0001$). The EFS in 60 mo by ISS was 38%, 32% and 10% for ISS I, II and III, respectively ($p< 0.0001$). The ANOVA showed significant difference for plasma cells bone marrow infiltration, creatinine and hemoglobin levels ($p< 0.0001$). The importance of ISS at diagnosis is emphasized due to its high capacity to discriminate among groups with low cost. In fact, the authors know that it is at the present moment too early to present any clinical trial results, for the protocol is still ongoing.

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CLINICAL AND EXPERIMENTAL RESEARCHES IN THE PLEURAL SPACE

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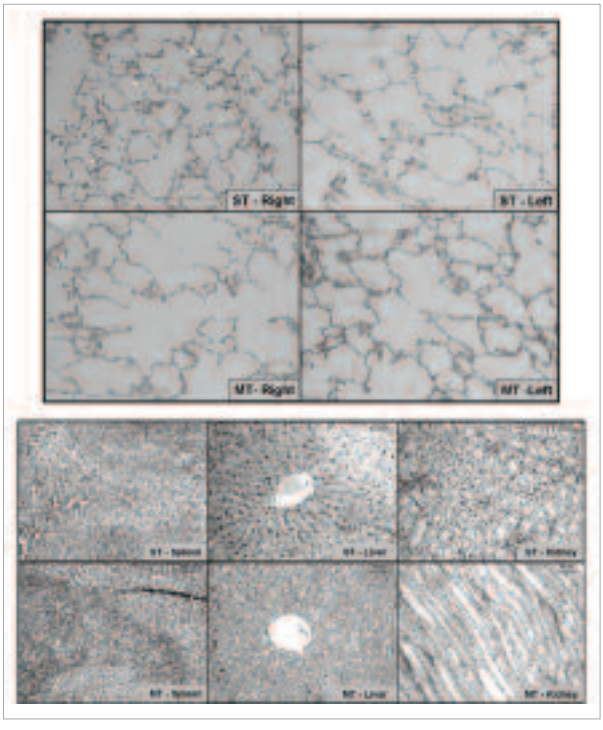


Presence of talc in lung and other organs after intrapleural injection of particles of different sizes. Small talc particles (ST) or of mixed size (MT). Infected hemithorax (right) or contralateral (left)

The objective is to characterize the response of the pleural cavity under several conditions: a) in humans, we will recognize the physiopathologic mechanisms that determine the accumulation of the pleural effusion and the clinical, emotional and functional participation; b) to know when the pleural fluid determines the beginning of the clinical symptoms and the recovery after the therapeutic procedures. The better understanding of the pleural inflammation mechanisms will allow for a better approach. The emphasis of this study is on tuberculosis and malignant pleural effusion, including epidemiologic studies and the pursue for new treatments. In addition, basic and experimental researches will allow to establish future research directions. The main focus is on pleurodesis by means of analyzing the sclerosing agents, morbidity and new treatment techniques.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The results of this study are divided in experimental, laboratory and clinical subprojects.

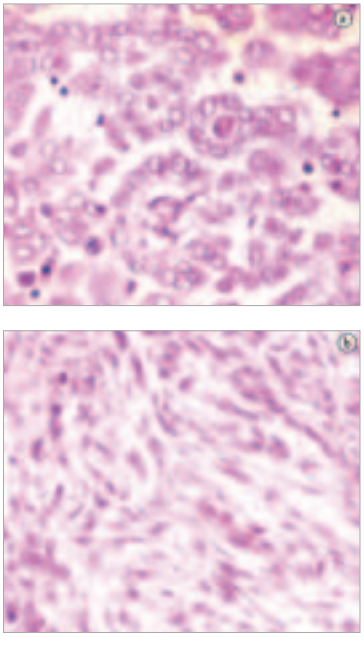
EXPERIMENTAL STUDIES

Intrapleural instillation of antibiotics (macrolides and quinolones) does not induce pleurodesis.

In talc pleurodesis there are important dispersions to several extrathoracic organs.

Small talc particles are responsible for a more pronounced systemic inflammation.

An effective participation of the mesothelial cells was characterized in the inflammatory process, and attributed to the intrapleural talc instillation.



Mesothelioma
A: malignant epithelial mesothelioma (HE x 200). B: malignant sarcomatoid mesothelioma (HE x 200)

LABORATORY ANALYSIS

The storage conditions up to 14 days do not interfere in the results of adenosine deaminase, proteins and glucose measured in pleural effusion.

The lactic dehydrogenase levels are more stable when stored at room temperature than in refrigerator or freezer.

CLINICAL STUDIES

Patients with moderate or massive pleural effusion show improvement in the walked distance (6 minutes), in FVC, FEV1 and in their sleep quality after therapeutic thoracentesis.

Patients with malignant pleural effusion can benefit from a pleurodesis induced with a small bore catheter.

In pleural tuberculosis, high levels of TGF in the fluid can be a predictive factor of pleural thickness.

In lymphocytic fluid, adenosine deaminase, proteins, the macroscopic aspect of the fluid and oncotic cytology are important diagnostic markers to differentiate between tuberculosis and cancer.

In infectious pleural effusions the cytokines can be considered modulators of the inflammatory process and markers of complications. There are high levels of VEGF, IL-1, IL-1ra and IL-8 in empyema and high levels of IL-10 in parapneumonic and in tuberculosis pleural effusion.

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PRO-INFLAMATORY MECHANISMS INVOLVED IN THE HYPOTHALAMIC CONTROL OF FEEDING AND THERMOGENESIS – IMPLICATIONS FOR THE PHISIOPATHOLOGY OF OBESITY, DIABETES MELLITUS AND CACHEXIA

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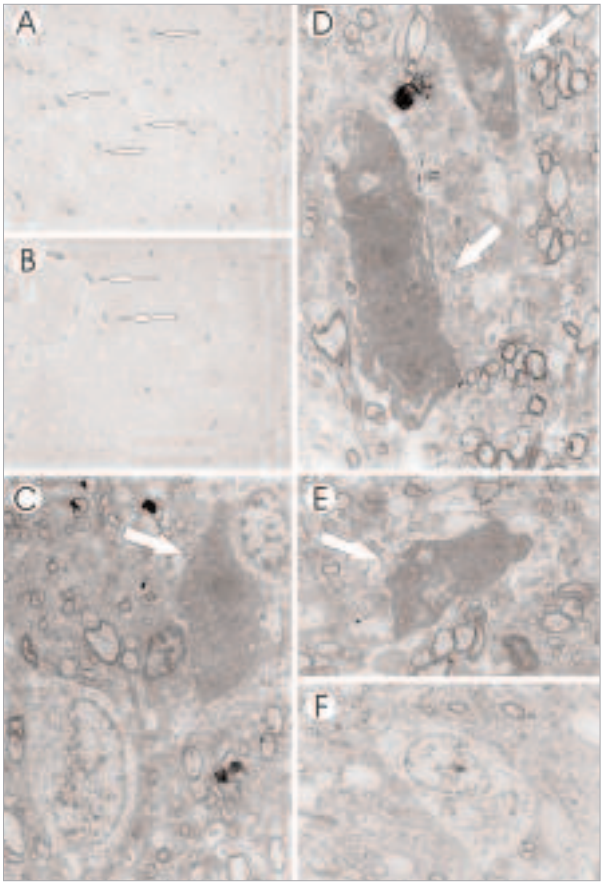

Electronic microscopy of apoptotic neurons in the hypothalamus of rats fed a high-fat diet (A-E). Non-apoptotic neuron from a control rat (F)

In the last decades there has been an astonishing increase in the prevalence of obesity and type 2 diabetes mellitus. Treatment based on modification of behavior and nutrition, and on the use of the few drugs available has not been sufficient to contain the advance of this epidemic. The characterization of the epithalamic mechanisms participating in the control of hunger and thermogenesis should allow for the identification of new targets for therapeutic approaches in these diseases. In the present project, multiple facets of the hypothalamic control of hunger and thermogenesis have been investigated. In the first stage, we carried out an analysis of the differential expression of RNAm in the hypothalamus of mice fed on a hyperlipidic diet. Modulations were detected in the mRNA expression of several inflammatory response proteins. In the next stage, we will investigate the role of TNF-α and IL-1β in the action of insulin in the hypothalamus and in the hypothalamic control of insulin secretion. We will further investigate the integration mechanisms between the hypothalamic signaling of IL-1β and insulin in a model of cancer.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

During the last 15 years obesity has become an epidemic phenomenon, affecting up to 30 % of the population of several regions of the world. As with other multifactorial diseases, the etiology of obesity is incompletely known. Multiple genetic loci have been identified as candidate regions encoding genes with potential role for the development of the disease, but some environmental factors, such as increased caloric intake and sedentary life style play an undisputed role in this context. The increased consumption of dietary fat is a hallmark of the modern occidental diet. Besides its intrinsic caloric value, dietary fats can act as inducers of inflammation in tissues such as muscle and liver. However, little was previously known about the role of dietary fats in the functional activity of the hypothalamus, the region of the brain responsible for controlling food intake and energy expenditure. The main objective of this project was to determine the effects of dietary fats on the function of the hypothalamus. For that we initially evaluated, by using a cDNA array, the effect of consumption of a fat-rich diet on the expression of genes in the hypothalamus. We found that 15% of the analyzed genes were regulated by the diet and that immune response genes were the most affected ones after functional clustering. Following that, we characterized the main cytokines expressed in the hypothalamus of rodents fed a high fat diet. This part of the study included the determination of several inflammatory and metabolic outcomes of the local actions of TNF-α and IL-1β. In addition, since dietary fats are complex and composed of several different types of fatty acids we evaluated the effects of isolated fatty acids in the hypothalamus. This study revealed that long chain saturated fatty acids are the most pro-inflammatory ones and that the inflammatory signal delivered by them depends on the recruitment of a specific receptor of the TLR family. At this moment we are engaged in the evaluation of the intracellular mechanisms that link the activation of TLR4 signaling with the control of the expression of inflammatory cytokines in the hypothalamus. Results obtained as part of this project may help find new targets for the treatment of obesity.

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