

## SESSIONS OF THE ACADEMIA BRASILEIRA DE CIÊNCIAS

### SUMMARY OF COMMUNICATIONS

#### CELL DAMAGE AND NEUROGENESIS IN THE DENTATE GRANULE CELL LAYER OF ADULT RATS AFTER PILOCARPINE- OR KAINATE-INDUCED STATUS EPILEPTICUS

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Presented by A.C.M. PAIVA

Dentate granule cells are generally considered to be relatively resistant to excitotoxicity and have been associated to robust synaptogenesis after neuronal damage. Synaptic reorganization of dentate granule cell axons, the mossy fibers, has been suggested to be relevant for hyperexcitability in human temporal lobe epilepsy and animal models. A recent hypothesis has suggested that mossy fiber sprouting is dependent on newly formed dentate granule cells. However, we have recently demonstrated that cycloheximide (CHX) can block the mossy fiber sprouting that would be otherwise induced by different epileptogenic agents and do not interfere with epileptogenesis in those models. Here, we investigated cell damage and neurogenesis in the dentate gyrus of pilocarpine- or kainate-treated animals with or without the co-administration of CHX. Dentate granule cells were highly vulnerable to pilocarpine induced-status epilepticus (SE), but hardly damaged by kainate induced-SE. CHX-pretreatment markedly reduced the number of injured neurons after pilocarpine-induced SE. Induction of SE dramatically increased the mitotic rate of KA and KA + CHX treated animals. Induction of SE in animals in-

jected with pilocarpine alone led to increases of between two to sevenfold in the mitotic rate of dentate granule cells as compared to increases of between five and thirtyfold for pilocarpine+CHX animals. These observations indicate that in presence of cycloheximide the increase of the mitotic rate after pilocarpine-induced SE may be due to protection of a vulnerable precursor cell population that would otherwise degenerate. We further suggest that the mossy fiber sprouting and neurogenesis of granule cells are not necessarily related events. — ( *September 14, 1999* ).

#### COMPETITIVE ANTAGONISM ASSOCIATED WITH BLOCKADE OF NEURONAL UPTAKE: EFFECTS OF INDORAMINE IN RAT VAS DEFERENS AND AORTA

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The  $\alpha 1$ -adrenoceptor antagonist indoramin was used in the rat vas deferens and aorta, against contractions induced by noradrenaline. Indoramin behaved as a competitive antagonist yielding  $pA_2$  values of  $7.38 \pm 0.05$  in rat vas deferens and  $6.78 \pm 0.14$  in aorta. In the presence of cocaine ( $6\mu M$ ), the potency ( $pA_2$ ) of indoramin in antagonizing the contractions of the vas deferens to noradrenaline was increased to  $8.72 \pm 0.07$  while its potency remained practically unchanged in the aorta ( $6.69 \pm 0.12$ ).

In denervated vas deferens, indoramin antagonized the contractions to noradrenaline with a potency similar to that found in the presence of cocaine ( $8.79 \pm 0.07$ ). It is suggested that indoramin blocks simultaneously  $\alpha_1$ -adrenoceptors and neuronal uptake in rat vas deferens, resulting in Schild plots with slopes not different from unity even in the absence of selective inhibition of neuronal uptake. As a major consequence of this double mechanism of action, the  $pA_2$  values for this antagonist are underestimated when calculated in situations where the neuronal uptake is active, yielding spurious  $pK_B$  values. — (September 14, 1999).

#### COMPARISON OF THE HUMAN IMMUNE RESPONSES TO RECOMBINANT PROTEINS REPRESENTING THREE DISTINCT SURFACE PROTEINS OF *Plasmodium vivax* MEROZOITES

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Presented by LUIZ R. TRAVASSOS

In the present study we evaluated the naturally acquired immune response to *Plasmodium vivax* Merozoite Surface Protein (MSP) 1, 3 and 4 in individuals with recent clinical episodes of malaria from the state of Pará, Brazil. Seven recombinant proteins representing different portions of MSPs were expressed in *E. coli* as GST fusion proteins. Immune responses were estimated in terms of frequency of responders, antibody titers, immunoglobulin isotype, longevity of the antibody response and cell mediated immunity. The total frequency of individuals with antibodies and cellular immune response to MSP1 was higher than to the other two proteins. The C-terminal region of MSP1 was the most antigenic as estimated by the frequency of responders and antibody titers. Neverthe-

less, the antibody responses to all antigens were relatively short lived. We concluded that MSPs were immunogenic to a certain proportion of individuals exposed to malaria. Our results also suggested that the C-terminal of MSP1 is particularly immunogenic to antibodies and T cells during natural infection in humans. — (September 14, 1999).

#### STRUCTURE AND TRANSCRIPTION OF GENES ENCODING THE SURFACE GLYCOPROTEIN ANTIGENS GP90 AND GP82 OF *Trypanosoma cruzi* METACYCLIC TRYPOMASTIGOTES\*

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Presented by LUIZ R. TRAVASSOS

The infective forms of *T. cruzi* are the trypomastigote stages found in the bloodstream of mammalian hosts or the metacyclic trypomastigotes present in the digestive tract of the insect. Metacyclic trypomastigotes express two stage-specific glycoproteins (gp90 and gp82) that have no counterpart in blood trypomastigotes [Teixeira & Yoshida 1986. *Mol Biochem Parasitol* **18**: 271-282]. The gp90 and gp82 are involved in the penetration of the parasite into host cells (Yoshida et al. 1990), [Ramirez et al. 1993. *Infect Immun* **6**: 3636-3641.], [Santori et al. 1996a. *Mol Biochem Parasitol* **78**: 209-216.]. Gp82 can induce  $Ca^{2+}$  signal in target cells [Dorta et al. 1995. *Mol Biochem Parasitol* **73**: 285-89.], [Ruiz et al. 1998. *Biochem J* **330**: 505-511.], an event essential for *T. cruzi* internalization (Dorta et al. 1995). Gp90 and gp82 are also relevant immunologically. Immunization with gp90 or gp82 protects mice against acute infection by *T. cruzi* [Yoshida et al. 1993. *Exp Parasitol* **77**: 405-413.], [Santori et al. 1996b. *Infect Immun* **64**: 1093-1099.].

cDNA clones encoding gp82 and gp90 were isolated from expression libraries using specific monoclonal antibodies [Franco et al. 1993. *Infect Immun* **61**: 4196-

4201.]; [Araya et al. 1994. *Mol Biochem Parasitol* **65**: 161-169.]. Comparison of sequences of gp90 and gp82 showed 40% identity at amino acid level, with homologous regions separated by sequences displaying significant amino acid differences (Franco et al. 1993; Araya et al. 1994). Sequence analysis of gp90 and gp82 also revealed 40–60% identity at amino acid level with members of *T. cruzi* gp85/sialidase family. Based on these structural features, gp90 and gp82 genes could be considered as members of gp85/sialidase family.

Large DNA fragments (40–400 kb) containing gp90 and gp82 sequences were isolated from *T. cruzi* genomic libraries constructed in YAC and cosmid vectors [Ferrari et al. 1997. *Mem Inst Oswaldo Cruz* **92**: 843-858.]. Gp90 and gp82 are present in multiple copies, distributed in several chromosomes, and this gene family can be divided into subsets on the basis of hybridization patterns obtained with probes derived from different regions of gp90 and gp82 genes (Araya et al. 1994); [Cano et al. 1995. *Mol Biochem Parasitol* **71**: 273-278.]; [Santos et al. 1997. *Mem Inst Oswaldo Cruz* **92**: 821-828.]. Many members of gp90 and gp82 gene family are closely linked to members of gp85/sialidase family at multiple sites in the genome of different *T. cruzi* strains. Hybridization patterns of gp90, gp82 and gp85 genes with *T. cruzi* chromosomal bands separated by pulsed field gel electrophoresis are very similar, suggesting that many of these genes could be linked in different chromosomal *loci*. This was confirmed by isolation of genomic DNA clones from YAC and cosmid libraries.

It is interesting to note that some several subtelomeric regions are made of sequences associated to the gp90 and gp85 [Chiurillo et al. 1999. *Mol Biochem Parasitol* **100**: 173-183.]. The presence of gp90 and gp85 at *T. cruzi* telomeres suggests that new variants of the gp85/sialidase family can continuously be arising by duplication, mutation, and recombination of copies that have been transposed to the telomeres.

Northern blot and western blot analyses showed that gp90 and gp82 are preferentially transcribed and expressed in the metacyclic trypomastigote stage (Franco et al. 1993; Araya et al. 1994). Further studies on the transcription of these genes using “run on” and RNA-PCR assays showed the presence of gp82 and gp90 transcripts in epimastigotes and blood trypomastigotes. Taken together these results suggest that the expression of genes gp90

and gp82 is constitutive and may be regulated at post-transcriptional level, for instance, at translational level and/or mRNA stabilization.

The presence of multiple copies of genes in *T. rangeli* encoding products related to *T. cruzi* gp82, gp90 and gp85 was revealed when genomic *T. rangeli* DNA was hybridized at moderate and high stringencies with gp82, gp90 and gp85 genes. Even at high stringency conditions, both probes hybridized with several genomic fragments suggesting that gp82, gp90 and gp85 related sequences are interspersed in the genome rather than arranged in tandem repeats. Sequence analysis showed that many of hybridizing fragments contain sequences associated to the gp85/sialidase gene family. These results suggested that gp90, gp82 and gp85 genes have been originated from a common ancestral gene present in several member of *Trypanosoma* genus. — ( *September 14, 1999* ) .

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#### SYNTHESIS AND IMMUNOLOGICAL ACTIVITY OF A BRANCHED PEPTIDE CONSTRUCTION CARRYING THE T CELL EPITOPE OF *Paracoccidioides brasiliensis* MAJOR EXOCELLULAR ANTIGEN, THE GP43\*

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The 43,000 kDa glycoprotein, gp43, is a major immunodominant antigen of *Paracoccidioides brasiliensis*. It has been used as a diagnostic reagent of paracoccidioidomycosis both in serological reactions and skin tests. The gp43 was cloned and completely sequenced [Cisalpinio et al. 1996. *J Biol Chem* **271**: 4553] and the structure of its single high-mannose carbohydrate chain determined [Almeida et al. 1997. *Glycobiology* **6**: 507]. Besides carrying B cell epitopes the gp43 also mediates cellular immune reactions [Rodrigues & Travassos 1994. *J Med Vet Mycol* **32**: 77]. Its T cell epitope was mapped to a 15-amino acid peptide (P10). Peptide 10 not only stimulates the in vitro lymphoproliferation of primed lymph node cells from sensitized or infected mice, but its immu-

nization with CFA in mice followed by an intra-tracheal challenge with a virulent strain of *P. brasiliensis* is protective, significantly reducing the number of colony-forming units in the lung and abolishing dissemination to the liver and spleen [Taborda et al. 1998. *Infect Immun* **66**: 786].

To increase the immunoprotection by P10, replace CFA as an adjuvant, and examine additional protocols including cytokines (e.g. IL-12), we are investigating different delivery systems for P10. Presently we synthesized an oligomeric multiple molecule derived from P10 and composed of four equal peptide chains attached to a three branched lysine core [LIAHTLAIRYAN)<sub>4</sub>-(K)<sub>2</sub>-K-G-amide, denoted M10]. Due likely to significant steric hindrance predicted during this parallel chain assembly, the initial synthesis attempt failed when the conventional protocol was applied. This occurred even when low substituted resin (methylbenzhydrylamine-resin, 0.2 mmol/g), recommended for minimizing chain aggregation inside resin beads and the common 50% dichloromethane (DCM)/dimethylformamide (DMF) solvent for crucial coupling steps were both used. Aiming to overcome this predicted difficulty, the synthesis protocol was altered following our previously proposed peptidyl-resin solvation approach [Cilli et al. 1996. *J Org Chem* **61**: 8992]. By applying this strategy, 20% dimethylsulphoxide/N-methylpiperidinone mixed solvent was alternatively used for coupling reactions throughout all tridecapeptide sequence growth. With this approach the target M10 was obtained although with low overall yield. A progressive decrease in the synthesis yield was observed as the chain length increased: 70%, 51% and only 27% for 1–5, 1–10 and 1–13 fragment syntheses, respectively). Despite this reduced final yield, we herein demonstrated that by applying the appropriate chemical strategy it is possible to synthesize extremely challenging macromolecules such as the lysyl branched-multiple tetrapeptide M10 composed of a total of 56 amino acid residues and reaching a molecular weight of more than 6 kDa.

Immunization of Balb/c mice with as little as 1 µg of M10 in CFA induced after 7 days a potent cellular immune response with lymphoproliferations being obtained in vitro with both M10 and P10 at 0.25 – 1.0 µg/ml. Immunization with 20 µg of P10 also elicited T lymphocytes proliferating with M10 and P10. On a molar basis the in vitro responses to 1.5 – 0.6 µM M10 were twice as intense as those to 6 – 3 µM P10. No proliferation

was observed with the truncated (IRYAN)<sub>4</sub>-(K)<sub>2</sub>-K-G-amide and (IHTLAIRYAN)<sub>4</sub>-(K)<sub>2</sub>-K-G-amide controls. These results suggest that the tetra-13 aa-peptide construction with oligomeric branching lysine (M10) can be used to elicit protective cellular immune responses against *P. brasiliensis*. — ( *September 14, 1999* ) .

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#### MONOCLONAL ANTIBODY SPECIFIC TO GLUCOSYL-CERAMIDE FOUND IN PATHOGENIC FUNGI

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Presented by L.R. TRAVASSOS

An IgG2a monoclonal antibody (MoAb) anti-glucosylceramide was established and termed MEST-2. HPTLC immunostaining, and solid-phase radioimmunoassay showed that MEST-2 reacts with glucosylceramides (GlcCer) from yeast and mycelium forms of *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, and *Sporothrix schenckii*; from mycelium forms of *Aspergillus fumigatus*; and from yeast forms of *Candida Albicans*, *Cryptococcus neoformans*, *Cryptococcus laurentii*, and *Cryptococcus albidus*. Indirect immunofluorescence showed that MEST-2 reacts strongly with the surface of yeast forms of *P. brasiliensis*, *H. capsulatum*, *S. schenckii*. Weak staining of mycelial forms of *P. brasiliensis* and hyphae of *A. fumigatus* was also observed. The sugar specificity of MEST-2 was assessed by inhibition assays using six different methyl-glycosides, and MEST-2 binding to GlcCer of the different fungi was inhibited only by methyl-β-D-Glc (80%). The lack of reactivity of MEST-2 with Gaucher's spleen GlcCer led us to analyze other structural features, besides β-D-Glc, that would be of importance in the reactivity of MEST-2 with fungal GlcCer. Studies on the fine specificity of MEST-2 using GlcCer purified from different strains of *Cryptococcus* and soybean showed that the 2-hydroxy group of the fatty acids is critical in the interaction of MEST-2 with fungal GlcCer. On the other hand, the importance of Δ<sup>3</sup> unsaturation of fatty acids, and the Δ<sup>4</sup> unsaturation of

the long chain base in the epitope recognized by MEST-2 were ruled out because soybean GlcCer, which presents the same reactivity of fungal GlcCer, does not present this structural feature. Also other structural features of fungal GlcCer such as the 9-methyl group and  $\Delta^8$  unsaturation of the sphingosine, apparently do not interfere the interaction between MEST-2 and fungal GlcCer since these structures are located far from the immunodominant epitope  $\beta$ -D-Glc and are possibly located in the lipid bilayer of the cell membrane. — (September 14, 1999).

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#### TOWARDS THE DISCOVERY OF MOLECULES INVOLVED IN HEMATOPHAGOUS BLOOD FEEDING

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*Triatoma infestans*, one of the most important vectors of Chagas disease is an exclusively hematophagous insect. When the insect bites the vertebrate's skin it injects a cocktail of molecules with a broad range of redundant pharmacological activities to avoid host hemostatic and inflammatory responses triggered by biting.

We have detected and characterized in *T. infestans* three anticoagulant activities; an inhibitor of platelet aggregation induced by ADP, epinephrine and thrombin; proteases; hemolytic and pore forming activities; a neuraminidase; an inhibitor of sodium channel; molecules capable of inducing ileum and uterus contraction and peroxidase and fosfatase enzymatic activity. Most of these molecules are present in the saliva and are injected in the host's skin. Some of them are synthesized as inactive molecules and activated when the saliva is released. This is the case of a novel salivary serine protease that we named Triapsin, which is activated by limited proteolysis at the moment of insect bite.

From the insect anterior midgut we purified, cloned and expressed a potent thrombin inhibitor, named Infestin, showing that anti-hemostatic activities are also present in the digestive tract of the insect, preventing blood coagu-

lation. The identification, characterization, cloning, and expression of these molecules in heterologous systems may provide new insights to understand anti-hemostatic mechanisms, the biology of hematophagous insect, and the discovery of novel molecules with pharmacological activities. — (September 14, 1999).

#### EFFECT OF $\beta$ -D-XYLOSIDES BEARING DIFFERENT AGLYCONES ON THE SYNTHESIS OF PROTEOGLYCANS DURING THE CELL CYCLE OF ENDOTHELIAL CELLS IN CULTURE

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PMA, an activator of PKC, stimulated the synthesis of heparan sulfate proteoglycan secreted to the medium of endothelial cells mainly during G<sub>1</sub> phase of the cell cycle (Porcionatto et al. 1998. *J Cell Biochem* **70**: 563-572). This result led us to investigate the effect of  $\beta$ -D-xylosides (that specifically stimulate the synthesis of proteoglycans) at different phases of the cell cycle. Glycosaminoglycan (GAG) biosynthesis initiates through the transfer of  $\beta$ -D-xylose from UDP-xylose to specific serine residues of the protein core. GAG biosynthesis can also occur on exogenous  $\beta$ -D-xylosides, but unlike endogenous xylosylated core proteins,  $\beta$ -D-xylosides preferentially stimulate chondroitin sulfate or dermatan sulfate synthesis and only weakly heparan sulfate or heparin synthesis (Schwartz et al. 1974. *Proc Natl Acad Sci* **71**: 40-51; Dietrich et al. 1982. *Biochim Biophys Acta* **717**: 478-485). Recently, it has been shown that the composition of glycosaminoglycans synthesized on  $\beta$ -D-xylose depends on the structure of the aglycone. Fritz & coworkers (1994. *J Biol Chem* **269**: 300-307) studying different aglycones linked to  $\beta$ -D-xyloside have shown that 2-naphthol- $\beta$ -D-xyloside (NX) and *cis/trans*-decahydro-2-naphthol- $\beta$ -D-xyloside (DX) were able to prime the synthesis of heparan sulfate chains in CHO cells. In the present study we compare the effect of NX, DX, p-nitro- and o-nitrophenyl- $\beta$ -D-xylosides as GAG acceptors in an endothelial cell line. It was observed stimulation of the synthesis of GAGs in a dose dependent manner. When cells were treated with 50 $\mu$ M NX for 15 hours there was a 6-fold increase in the amount of heparan sulfate (HS) and a 19-fold in-

crease in the amount of chondroitin sulfate (CS) secreted to the medium, reaching a plateau with  $100\mu\text{M}$  (9- and 26-fold, respectively). On the other hand, no differences in the amounts of HS from the cell extract were observed between control and treated cultures, whereas for CS a 9-fold increase ( $50\mu\text{M}$ ) and 12-fold increase ( $100\mu\text{M}$ ) were observed. The results obtained for the two different isomers of nitrophenyl- $\beta$ -D-xylosides show that the compounds displace different dose response curves. The optimum concentration for enhancement of the GAG synthesis was  $500\mu\text{M}$  for p-nitrophenyl and  $100\mu\text{M}$  for the o-nitrophenyl derivative. For the p-nitrophenyl there was a 7-fold increase in the amount of HS and a 26-fold increase in the amount CS secreted to the medium. The amount of CS present in the cell was increased 4 fold. For the o-nitrophenyl there was a 20-fold increase in the amount of HS and a 100-fold increase in the amount CS secreted to the medium. The amount of CS present in the cell was increased 6 fold. On the other hand, no differences in the amounts of HS from the cell extract were observed between control and treated cultures with both isomers. The CS synthesized in the presence of the xylosides shows a different electrophoretic migration (more dermatan sulfate-like) when compared to the ones from control cultures. To study the effect of these compounds on the cell cycle, the cells were submitted to 24 hours of starvation and then stimulated to proliferate with the addition of fetal calf serum (FCS) in the absence or in the presence of  $\beta$ -D-xylosides in different periods of time. NX, DX and p-nitrophenyl did not alter the cell cycle whereas the mitogenic effect of FCS on the cells was abolished after treatment with o-nitrophenyl ( $75\mu\text{M}$ ). These results indicate that hydrophobicity of the aglycones linked to the  $\beta$ -D-xylosides is not the only factor involved in priming of the GAGs. Thus the o-nitrophenyl

(less hydrophobic than NX and DX) is the best acceptor for HS synthesis and curiously was the only compound capable of interfering with the cell cycle, blocking the cell entrance in the S phase. — ( *September 14, 1999* ).

\* Supported by: FAPESP and CNPq.

## ERRATA

### **Isótopos do Carbono dos Carvões e da Matéria Orgânica do Solo em Estudos de Mudança de Vegetação e Clima no Quaternário e da Taxa de Formação de Solos do Estado de São Paulo**

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By a lapse, Figs. 1 and 2 were reversed. In addition, mention to grant FAPESP 96/12777-2 was omitted from the Acknowledgments section. We apologize for any inconvenience arising from these errors.

### **Isótopos do Carbono dos Carvões e da Matéria Orgânica do Solo em Estudos de Mudança de Vegetação e Clima no Quaternário e da Taxa de Formação de Solos do Estado de São Paulo**

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An Acad Bras Ci 71(4-II) 969-980.

Devido a um equívoco, as figuras 1 e 2 foram revertidas. Além disso, foi omitida a menção ao auxílio FAPESP 96/12777-2 na seção de Agradecimentos. Pedimos desculpas por qualquer inconveniência decorrente destes erros.