Latin America multidisciplinary research on heat shock proteins and cell stress: proceedings of the first conference of the Latin America Chapter of the Cell Stress Society International

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Abstract The First Conference of the Latin America Chapter of the Cell Stress Society International (CSSI) organized by CSSI was held in Montevideo, Uruguay, on March 11–14, 2014. The Latin America Chapter of the CSSI (LAC-CSSI) was established at the Workshop on the Molecular Biology of the Stress Response, Porto Alegre, Brazil, May 2012. The chapter's first meeting took place in the beautiful city of Montevideo and was chaired by the first (LAC-CSSI) elected president Professor María Bausero. Forty-two invited speakers presented their work to more than 100 scientists. The first day of the conference was dedicated to an introductory program for students, young investigators, and participants new to the field of molecular chaperones and the stress response. These seminars were held in the Pasteur Institute of Montevideo and the Faculty of Sciences of the University of the Republic. These institutions were carefully selected to give foreign participants a broad view of the diversity of students and institutions doing research in Uruguay, as well as an opportunity for direct interaction with our scientists and students. Invited speakers for the seminar series were Dr. Wolfgang Schumann, Dr. Cristina Bonorino, Dr. Antonio De Maio, Dr. Ian Brown, Dr. Rafael Radi, Dr. Daniel Ciocca, and Dr. Celia Quijano. The remaining days of the conference took

place at the Sheraton Hotel in Montevideo, and the scientific sessions are discussed below.

Keywords First Conference of the Latin America Chapter · Cell Stress Society International · Molecular chaperones · Heat shock proteins · Cellular stress responses

Introduction

During the last 10–15 years, tremendous efforts have been made by governments in Latin America to reverse earlier restrictions on scientific research and to support research on heat shock proteins, chaperones, and cell stress. The Cell Stress Society International (CSSI) is at the vanguard of international scientific organizations in creating innovative strategies to support this research. From its inception, the CSSI has held workshops alternately between third world and industrialized countries, thus strengthening collaborations and narrowing the gap between scientific communities.

Consequently, there has been an exponential increase in the number of Latin American heat shock proteins and chaperones researchers participating at these meetings, laying the ground for the establishment of the Latin America Chapter (LAC-CSSI) of the Cell Stress Society International at the Porto Alegre Meeting in May 2012 in Brazil. Two years later, the First Conference of the LAC-CSSI was held in Montevideo, Uruguay in March 2014 (Fig. 1).

Close to 100 participants arrived from 10 different nations including Mexico, Brazil, Argentina, Colombia, and Chile, in addition to participants from Europe, the USA, Canada, and Asia, which stimulated interactions among researchers from “both worlds.” The unexpectedly varied sessions included translational medicine and agricultural sciences, health, and fundamental research. The first day was dedicated to courses

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Fig. 1 Poster Award Session at Montevideo 2014: Left to Right Josielle Abrahão (Ramos lab), Larry Hightower (Session Chair), Jéssica F. Araujo (Bonorino lab), Maria Bausero (Meeting Organizer), Antonio De Maio (CSSI Ambassador) and Thiago Seraphim (Borges lab). Award winner not present: Mayra Sottile (Nadin lab)

for students and researchers new to the field and took place at the Institute Pasteur of Montevideo and at the Faculty of Sciences at the University of the Republic. The courses and seminars were given by Latin American professors (Drs. Cristina Bonorino, Antonio de Maio, Rafael Radi, Daniel Ciocca, and Celia Quijano) and by Drs. Wolfgang Schumann and Ian Brown). The conference was opened with remarks from Dr. Larry Hightower on the history of the CSSI and from Dr. Antonio De Maio on the events that led to the creation of the Latin America Chapter. The talks covered very diverse fields on cell stress and chaperones and are discussed below. Each session covered topics of particular relevance to Latin American researchers such as infectious diseases, agriculture, cancer, translational medicine, and fundamental research.

Cellular responses to oxidative stress

This first session focused on metabolic oxidative stress.

Maria Clara Franco (Burnett School of Biomedical Sciences, Florida, USA) Maria Clara Franco discussed the role of nitrated Hsp90 on mitochondrial metabolism. Peroxynitrite production and nitrotyrosine formation occur in several pathologies, including cancers. However, whether tyrosine nitration plays a role in the regulation of cancer cell metabolism is unknown. We found that site-specific nitrated heat shock protein 90 (Hsp90) associates with mitochondria and downregulates mitochondrial activity in cancer cells, a hallmark of cancer energetic metabolism. Endogenously nitrated Hsp90 colocalizes with mitochondria in vivo in breast cancer tumors. Because nitrated Hsp90 is not detected in normal cells and regulates the energetic requirements of cancer cells, it constitutes an exceptional target to develop drug therapies focused specifically on tumor cells.

Carlos Robello (Institut Pasteur de Montevideo, Molecular Biology Unit, Montevideo, Uruguay) Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* that has the peculiarity to invade almost any type of cell. The parasite interacts, through its surface, with host-surface molecules, in order to generate signaling and/or metabolic changes that favor infection. This work focused on the early response of human cells to *Trypanosoma cruzi* infection, in epithelial cells, macrophages, and cardiomyocytes, through the study of transcriptomic changes in response to infection. In the three cell types studied, hundreds of genes are upregulated and downregulated immediately after infection: cells are literally reprogrammed. However, a detailed analysis of the altered pathways clearly indicates that each cell type has extremely different responses. Epithelial cells are mostly altered in pathways related to inflammatory and lipid metabolism genes; in cardiomyocytes, the glycolysis and oxidative phosphorylation are the most affected pathways, whereas in macrophages albeit immune response-related genes, the most upregulated genes are related to autoimmune diseases. In summary, this intracellular pathogen generates a cell stress response that can be considered as a reprogramming that favors parasite persistence.

Andrés Trostchansky (Departamento de Bioquímica-CEINBIO, Facultad de Medicina, UdelaR, Montevideo, Uruguay) Nitro-fatty acids (NO₂-FA) are electrophilic signaling mediators inducing a variety of cytoprotective and anti-inflammatory pathways, e.g., through modulation of gene transcription. Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by the progressive loss of motor neurons, where the activation of the transcription factor Nrf2 in astrocytes confers protection to neighboring neurons. In this talk, Andres presented data on the evaluation of the effects of NO₂-FA on an in vitro model of motor neuron degeneration induced by astrocytes expressing the ALS-linked SOD1^{G93A} mutation. NO₂-FA induce hemoxygenase-1 expression in

astrocytes via Nrf2 activation while a transient exposure of SOD1^{G93A} astrocytes to NO₂-FA prevented their toxicity to motor neurons, supporting a novel neuroprotective role

Florencia Arredondo, (Neurochemistry Dept., IIBCE-MEC, Montevideo, Uruguay) Pharmacological interventions that modulate the NF-E2-related factor 2 (Nrf2) and its downstream phase II ARE genes are promising targets for the treatment of several neuropathologies because of their potent ability to detoxify cell stressors and to maintain redox homeostasis. In her talk, Arredondo described the modulation of Nrf2-dependent endogenous defense systems by the flavonoid quercetin and proposed that this mechanism could be involved in its neuroprotective effect, suggesting a protective role for the flavonoid in several pathophysiological states including neurodegeneration

Cell stress in plants

This field was specially introduced in Montevideo since the economies of many Latin American countries depend on crop production. Thus, how plant cells cope with heat and abiotic stress is of increasing interest among plant biologists and crop producers.

Estela Valle (Instituto de Biología Molecular y Celular de Rosario (IBR), Rosario, Argentina) Estela Valle's talk focused on small heat shock proteins and the cold stress response in tomato fruit. Shelf life, one of the most important agronomic traits for tomatoes, is lengthened by storing fruit at low temperature. Susceptible fruits suffer physiological disorders known as chilling injury. It has been reported that mitochondrial small heat shock proteins (MT-sHSP) were induced after cold storage of tomato fruits; thus, MT-sHSP23.8 is involved in the response of tomato fruit to cold stress, which could have implications for developing tomato cultivars tolerant to environmental stress.

Lorenzo Lamattina (Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina) Lorenzo Lamattina discussed the regulation of abscisic acid (ABA) and nitric oxide (NO) in response to ultraviolet radiation (UV-B). These elements regulate a number of well-characterized signaling pathways in plant responses to environmental stresses. They propose that the induction of common signaling components including ABA, NO, and Ca²⁺ orchestrates adaptive responses to high doses of UV-B, suggesting that the evolution of a general mechanism activated by UV-B is conserved in multicellular organisms challenged by a changing common environment.

Eduardo Ceccarelli (Instituto de Biología Molecular y Celular de Rosario (IBR) CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina) Eduardo Ceccarelli described how the Clp system interacts with the stress response. *Arabidopsis thaliana* shows a remarkable diversity of Clp proteins. They found that ClpC2 and D are homodimers in solution, and upon addition of ATP, they assemble into hexamers. Both proteins exhibited basal ATPase activity with distinctive *k*_{cats} and *K*_ms, indicating different metabolic roles. They have evidence that ClpS functions as a modulator of the Clp system, recognizing substrates following an N-end rule.

Sabina Vidal (Laboratory of Plant Molecular Biology, Faculty of Science, UdelaR, Montevideo, Uruguay) Dr. Vidal's group focused on gene gain of function during abiotic stress tolerance in the model of *Physcomitrella patens*. Plant genomes that are highly adapted to specific types of abiotic stress are an interesting source of genes for improving stress tolerance in cultivated species. The moss *P. patens* can withstand extreme environmental conditions including drought and salt stress. In this talk, the authors reported on the functional characterization of two types of abiotic stress-upregulated genes, the dehydrins PpDHNA and PpDHNB, and a small heat shock protein PpHsp16.4. Both types of proteins are part of a well known and conserved cell protecting mechanism in plants. This work provides the first direct genetic evidence for the role of an sHsp in osmotic and salt stress tolerance and supports a function for this protein particularly during the stress recovery stage of *P. patens*.

Cell stress and chaperones

An introduction to the topic was given by *Harm Kampinga (Department of Cell Biology, Groningen, The Netherlands)* who discussed the role that chaperones have on protein folding diseases. Numerous human diseases are associated with the formation of toxic protein aggregates. Kampinga summarized the existing literature on a set of aggregation diseases, each by a different set of HSP with specific aggregation types.

Alejandra Erlejamn (Dept. Biological Chemistry-IQUIBICEN, School of Natural Sciences, Univ. of Buenos Aires, Buenos Aires, Argentina) Nuclear factor κ B (NF- κ B) is a transcription factor involved in stress-induced, immune, and inflammatory responses. Canonical NF- κ B is an inactive heterodimer of p65/RelA and p50 subunits located in the cytoplasm bound to I κ B. NF- κ B activation causes I κ B phosphorylation, its dissociation, and NF- κ B nuclear translocation. Because FKBP51 and FKBP52 modulate both trafficking and transcriptional activity of steroid receptors, the authors

investigated if FKBP51 and FKBP52 are also able to modulate NF- κ B properties as they do with steroid receptors. Here, they showed the first evidence that the balance of the cell expression for both immunophilins modulate NF- κ B function in an opposite manner, and that both immunophilins show regulatory action on transcription due to their selective recruitment to promoter regions of NF- κ B target genes.

Mario Galigniana (Dept. of Biological Chemistry, University of Buenos Aires, Buenos Aires, Argentina) Hsp90 is the major soluble protein of the cell. Hsp90 is a homodimer, and each protomer contains three flexibly linked regions: the NT-domain or ATP-binding domain, the M-domain, and the CT-domain or dimerization domain. The latter also shows a conserved MEEVD motif that serves as the docking site for cochaperones via a tetratricopeptide repeat (TPR) clamp. In this talk, Galigniana discussed the biological relevance of the Hsp90-TPR protein stoichiometry on several models such as the functional regulation of steroid receptors, the telomerase activity in tumor cells, the nuclear retention of transcription factors by the nucleoskeleton arrangement, and molecular events related to the neurodifferentiation process. In all of them, the biological properties shown by the chaperone heterocomplexes in the cell support the existence of a single TPR protein associated to Hsp90 dimers, which can be dynamically exchanged by other TPR protein on mutually exclusive fashion.

Carlos Ramos (Dept. Organic Chemistry, Univ. of Campinas UNICAMP, Campinas, Brazil) All members of the Hsp100 chaperone family are unique in the sense that they contain a distinct disaggregase activity, which is capable of clearing toxic protein aggregates from the cell. Although the most studied eukaryotic Hsp100 member is Hsp104 from yeast, *Saccharomyces cerevisiae*, clear orthologs have been identified in bacteria, plants, and some protists, but not in metazoans. Results were discussed showing that a human protein complements the phenotype of knocked-out yeast under stressful conditions such as thermal stress.

Graciela Piwien (Instituto de Biología y Medicina Experimental, Buenos Aires, Argentina) Obesity is a serious health problem of universal concern. Graciela Piwien's novel presentation focused on adipocyte differentiation and the nuclear lamina reorganization. Obesity is, in part, the result of increased adipogenesis so that it is important to discover how this process is controlled. This talk emphasized the role that Hsp90-binding cochaperone FKBP51 plays during the reorganization of the nuclear lamina and the control of gene expression during the process of adipocyte differentiation. When 3T3-L1 preadipocytes differentiate, FKBP51 expression progressively increases while FKBP52 decreases. At the onset of

adipogenesis, FKBP51 transiently translocates from mitochondria to the nucleus. FKBP51 mitochondrial-nuclear shuttling depends on PKA signaling. In summary, PKA-dependent mitochondrial-nuclear translocation of FKBP51 possibly coordinates the reorganization of the nuclear lamina required for the acquisition of adipocyte phenotype.

Cell stress and cancer

Gabriella Santoro (Dept. of Biology, University of Rome, Rome, Italy) Dr. Santoro's talk served as the background to understanding how Hsps and inflammation interact and play a major role in tumor biology.

The heat shock response (HSR) represents a fundamental protective mechanism utilized by mammalian cells to preserve cellular function and homeostasis under stress conditions which implies the activation of the HSF-1 transcription factor. In cancer cells, however, activation of HSF1 has been associated with both antiapoptotic and proapoptotic responses. Antiapoptotic HSP expression generally results in cancer cell thermotolerance and chemoresistance; therefore, disruption of HSF1 signaling is considered therapeutic in several types of tumors. On the other hand, HSF1 activation may result in apoptosis induction in aggressive cancers presenting aberrations in survival signaling dependent on the proinflammatory factor NF- κ B. Here, Santoro described the proapoptotic activity of a new class of potent HSF1 inducers, and discussed how, in addition to heat shock proteins, HSF1 and HSF2 regulate the expression of proteins with nonchaperone function involved in survival and inflammatory signaling.

Silvina Nadin (Institute of Medicine and Experimental Biology of Cuyo, National Scientific and Technological Research Council of Argentina, Mendoza, Argentina) Proposed a possible mechanism by which some Hsps (HSPB1, HSPA1A), HSF-1 and MLH1, MSH2 interact with proteins of the mismatch repair protein system (MMR). There is increasing evidence that HSPs participate in DNA repair mechanisms. Cisplatin is a widely used anticancer agent, and resistance to the drug has been connected with mismatch repair (MMR) deficiency. MMR protein complexes (MSH2/MSH6, MLH1/PMS2) are able to bind cisplatin-DNA, important for drug-mediated cytotoxicity. Silvina Nadin's work characterized the effects of cisplatin and hyperthermia on cell viability, on HSPB1, HSPA1A, HSF1, MLH1, MSH2, protein expression and demonstrated associations between HSPs and MMR proteins suggesting for the first time that MLH1 and MSH2 are client proteins of HSPA1A and HSPB1.

Niubys Cayado-Gutierrez, (Oncology Laboratory, Institute of Experimental Medicine and Biology of Cuyo, Regional Center for Scientific and Technological Research, National Research Council (CONICET), Mendoza, Argentina) Examined the role of HspB1 phosphorylation on PTEN and proteins related to breast cancer progression such as β -catenin, caveolin-1, NHERF1, and HER2/neu. They found that HSPB1 phosphorylation modulates PTEN inactive form as well as proteins associated with tumor progression..

Juan Iovanna (Centre de Recherche en Cancérologie de Marseille (CRCM), Marseille, France) Pancreatic ductal adenocarcinoma (PDAC) progresses from precursor lesions named pancreas intraepithelial neoplasias (PanINs). In this regard, it has been firmly established that oncogenic mutations in *KRAS* behave as one of the earliest stimuli for the formation of PanINs. It is noteworthy, however, that during the initiation stage, pancreatic cells not only trigger protumoral processes, but also cellular events that counteract transformation. One of these tumor suppressive processes, elicited by Kras activation, is cellular senescence (oncogene-induced senescence or OIS). The nuclear protein 1 (Nupr1) is strongly induced by pancreatitis and several other cell stresses. The fundamental role that Nupr1 plays in pancreatic tumorigenesis is underscored by recent results presented by Dr. Iovanna showing that, in mice, the oncogenic form of *Kras*^{G12D} is unable to promote PanINs in absence of this chromatin protein. His group identified the molecular mechanisms by which Nupr1 influences Kras-mediated preneoplastic growth. In fact, they find that genetic inactivation of Nupr1 in mice impairs Kras-induced PanIN leading to an increase in β -galactosidase-positive cells and upregulation of surrogate marker genes for senescence. More importantly, both of these cellular and molecular changes are recapitulated by the results of mechanistic experiments using RNAi-based inactivation of Nupr1 in human pancreatic cancer cell models. In addition, the senescent phenotype, which results from Nupr1 inactivation, is accompanied by activation of the FoxO3a-Skp2-p27^{Kip1}-pRb-E2F pathway in vivo and in vitro. Finally, activation of the oncogenic Kras into the lung induces formation of adenomas in Nupr1 wild-type mice as expected whereas it induces OIS in Nupr1-deficient mice. Nupr1 is one, if not the only, stress-induced factor participating in the oncogene-induced transformation. This new knowledge is medically relevant as it delineates early pathobiological events that may be targeted in the future as a means to interfere with the formation of preneoplastic lesions early during pancreatic carcinogenesis.

Vanessa Gottifreddi (Fundación Instituto Leloir, Buenos Aires, Argentina) Vanessa Gottifreddi discussed how proliferating cells deal with replicating damaged DNA. During UV irradiation, replicative DNA polymerases are blocked by UV-triggered DNA lesions and cells activate tolerance

mechanisms to avoid the permanent stalling and collapse of replication forks. The best characterized tolerance mechanism is translesion DNA synthesis (TLS), a pathway that utilizes alternative DNA polymerases capable of using DNA lesions as replication templates. Another tolerance mechanism that might contribute to DNA replication after UV damage is template switching. This pathway utilizes the newly replicated DNA in the sister chromatid as an alternative template. While TS is not triggered by double-strand breaks (DSBs), it requires the participation of classic homologous recombination (HR) factors. The contribution of TS to the replication of UV-triggered DNA lesions is yet poorly characterized. Nucleolytic degradation of newly synthesized DNA and the reversal of replication forks might also have an impact on the speed of fork elongation when DNA is damaged. Thus, the data here presented suggest a strong collaboration of TLS and HR factors in the elongation of replicating forks after UV irradiation.

Cell stress and neurosciences

Ian Brown (Center for the Neurobiology of Stress, University of Toronto, Toronto, Canada) Neurodegenerative diseases like Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (ALS) have been characterized as protein misfolding disorders in the brain. Heat shock proteins (Hsps) are protein repair agents that provide a line of defense against misfolded, aggregation-prone proteins. Celastrol, a traditional compound used in traditional Chinese medicine, induces the expression of Hsps, including Hsp70B', in human neuronal cells. In vivo administration of celastrol in a mouse model of Alzheimer's reduces aggregation of amyloid-beta protein, suggesting that it could be useful as a neuroprotective drug. Arimoclomol is an experimental drug currently under clinical trial for the treatment of ALS that is a coinducer of the Hsps. Brown demonstrated that coapplication of low dosages of celastrol and arimoclomol, at levels that do not affect cell viability, enhanced the induction of Hsps, including Hsp70B', in human neuronal cells.

Alvaro Estevez (Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Florida, USA) In robust work presented at this conference, the Estevez research group identified 17 proteins that are major targets for peroxynitrite. This is relevant because production of peroxynitrite and tyrosine nitration is associated with all neurodegenerative diseases, central nervous system trauma, and stroke. Peroxynitrite induces apoptosis by a mechanism involving nitration of tyrosine residues. Nitration of a single tyrosine residue on 2–4 % of the prosurvival chaperone heat shock protein 90 (Hsp90) activated a toxic gain-of-function that induced motor neuron and PC12 cell death. Here, they demonstrate that nitration of a single tyrosine

residue in Hsp90 is necessary and sufficient to trigger a toxic gain-of-function that results in cell death by activation of an apoptotic pathway.

Claudio Hetz (Biomedical Sciences Institute, University of Chile, Santiago, Chile) The most common neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease, affect millions of people worldwide, and there is neither preventive nor curative therapy for them. These diseases share a common neuropathology, primarily featuring the presence of abnormal protein inclusions containing specific misfolded proteins. Recent evidence indicates that alteration in organelle function is a common pathological feature of protein misfolding disorders. The endoplasmic reticulum (ER) is an essential compartment for protein folding, maturation, and secretion. Signs of ER stress have been extensively described in most experimental models of neurological disorders. ER stress is caused by functional disturbances, which result in the accumulation of unfolded/misfolded proteins at the ER lumen. To cope with ER stress, cells activate an integrated signaling response termed the unfolded protein response (UPR), which aims to re-establish homeostasis through transcriptional upregulation of genes involved in protein folding, quality control, and degradation pathways. This talk gave an overview of efforts to assess the role of ER stress in protein misfolding disorders and discussed possible strategies to target the UPR with therapeutic benefits.

Patricia Cassina (Facultad de Medicina, Universidad de la República, Montevideo, Uruguay) Astrocyte-mediated neurotoxicity to motor neurons is a recently recognized pathogenic mechanism in amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases. Induction of mitochondrial dysfunction in astrocytes by different stimuli including the expression of ALS-linked SOD mutations leads to an inflammatory astrocytic phenotype that promotes the death of neighboring motor neurons. Importantly, these deleterious effects are prevented by the mitochondrially targeted antioxidants, ubiquinone, or carboxy proxyl nitroxide covalently coupled to a triphenylphosphonium cation (MitoQ and Mito-CP, respectively) suggesting that defective mitochondrial respiration is associated with nitroxidative stress. In addition, dichloroacetate (DCA), which is known to shift pyruvate metabolism from glycolysis toward oxidation in the mitochondria, also prevents glial-mediated neurotoxicity. Patricia Cassina's group performed pharmacological interventions directed to the mitochondria in ALS mice and studied motor neuron symptoms in order to clarify the role of mitochondrial

dysfunction in ALS progression. Understanding how mitochondria modulate the astrocyte-derived neurotoxicity will shed light onto novel approaches for ALS therapy with outstanding potential for clinical development.

Cell stress and infectious diseases

Bertha Espinoza (Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico) Trypanosomacruzi, the causative agent of Chagas Disease, needs to adapt to different conditions during its life cycle. This includes different types of stress. The adaptation to these conditions is important for survival, reproduction, and transmission of the parasite. In this work, different types of stress were analyzed. Among them were nutritional, acidic, oxidative, and thermal stress. Each type of stress produced differential effects on the parasite, both at the protein as well as at the structural level. Proteomics studies as well as electron microscopy analysis showed effects in the mitochondria and cell death due to autophagy. Interestingly, when the two main stages of the parasite were compared with respect to their response to the different types of stress, they showed different behavior, with the epimastigotes being more affected than the trypomastigotes. In addition, a small heat shock protein (sHsp) was characterized for the first time in *T. cruzi*. This sHsp was determinate to be of 16 kDa. When different stress conditions were analyzed, this protein was upregulated only by thermal and oxidative stress. This phenomenon was observed only in the epimastigotes stage and not in the trypomastigotes stage, indicating a regulated adaptive response.

Mariana Boiani (Dept Biochemistry, Faculty of Medicine, UDELAR, Montevideo, Uruguay) Reduced BAG3 expression during *T. cruzi* infection may modulate cardiomyocyte apoptosis.

Iara Linhares (Visiting Professor-Dept. of Obstetrics and Gynecology FMUSP, San Pablo University, San Pablo, Brazil) This talk showed the evaluation of the presence of heat shock proteins as biomarkers of female genital tract infections. Bacterial vaginosis (BV) is a syndrome in which *Lactobacilli* are replaced in the vagina by an overgrowth of anaerobic bacteria. This is associated with an increase in susceptibility to upper genital tract infections and, in pregnant women, with a higher rate of preterm birth. They showed that detection of the 70-kDa heat shock protein (hsp70) in the vagina was a biomarker for the presence of BV. In another study, they demonstrated that a fallopian tube infection with *Chlamydia trachomatis* leads to the persistent release of the chlamydial 60-kDa heat shock protein (hsp60). Since the chlamydial hsp60 is 50 % homologous to the human hsp60, a

chlamydial tubal infection leads to development of autoantibodies to the human hsp60. The human hsp60 is one of the first proteins expressed by the human embryo, and so, autoimmunity to hsp60 inhibits fertility.

Cell stress and immunology

Alfonso Barreto (Dept. of Microbiology, Science School, Pontificia Universidad Javeriana, Bogotá, Colombia) Rotavirus (RV) predominantly replicates in intestinal epithelial cells. The authors used Caco-2 cells as a model to study danger signals produced during RV infection. RV infection induces the release of Hsc70, Hsp70, and TGF- β , in part associated to extracellular vesicles (EVs). They detected the presence of exosomes and apoptotic bodies in these EVs, using appropriate markers and obtaining them in the presence or absence of caspase inhibitors. These EVs are associated with a small part of virus antigen, which could be used in antigen transfer mechanisms during the induction of immune response. On the other hand, EVs decrease viability and proliferative response of polyclonally stimulated T CD4⁺ cells throughout the expression of TGF- β . We propose that during infection, RV increase a heterogeneous population of EV composes in part by exosomes and apoptotic bodies which could be responsible for an increased tolerogenic response in the intestine.

Jose Alexandre Barbutto (Dept. of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, SP, Brazil) Jose Alexandre Barbutto addressed the interrelation of HSPs and dendritic cell (DC) maturation in cancer patients. Monocytes from breast cancer patients showed a distinct pattern of activation, including a different pattern of suppressors of cytokine signaling, before any differentiation signal was delivered to them. Upon differentiation, the ensuing monocyte-derived DCs (Mo-DCs) showed increased levels of Hsp27 mRNA, but not intracellular Hsp27 protein. In addition, these Mo-DCs presented an enhanced ability to induce regulatory T cells (CD4⁺CD25⁺Foxp3⁺) in vitro. Mo-DCs from healthy donors, treated with breast cancer cell line supernatant, presented phenotypic alterations and increases in pp38 and pHsp27. These data indicate that cell stress upon monocytes and DCs has a complex response pattern in cancer patients that needs to be better characterized.

Thiago Borges (Laboratory of Cellular and Molecular Immunology, Biomedical Research Institute, Pontificia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil) Thiago Borges showed how mycobacterial DnaK impairs dendritic cell capacity to induce alloreactive T cell responses. Heat shock proteins (Hsps) act intracellularly to protect cells from damage; however, they can also be secreted and have immune modulatory effects. DnaK

from *Mycobacterium tuberculosis* belongs to the Hsp70 family. In the present study, we tested the effects of DnaK on fully differentiated DCs and on its capacity to stimulate alloreactive T cells. We observed a decreased number of proliferating alloreactive T cells when they were cultured with DnaK-treated DCs. Finally, we treated skin allografts with DnaK or OVA and evaluated T cell proliferation in draining LNs from host mice. The total number of LN cells was reduced in mice that received DnaK-treated grafts. Additionally, after 24 or 96-h posttransplantation, we observed decreased CD8⁺ and CD4⁺ T cell proliferation in these mice. These results suggest that DnaK treatment impairs the stimulatory ability of DCs to stimulate alloreactive T cells, through the downregulation of MHC II and CD86 on DCs. This is a potent innate effect that probably plays a role in immune evasion in vivo, and yet can be harnessed to manage graft rejection.

Cell stress and translational research on medicine

Translational research aims to shorten the time needed for a therapeutic agent to be released for clinical application. Heat shock proteins and cell stress research are not excluded from these new disciplines. This is the first meeting at which we included a special session on translational medicine.

John Williams (Chester Centre for Stress Research, University of Chester, Chester, UK) John Williams gave an opening introduction to extracellular Hsps, and applied research on cancer, cardiovascular diseases and diabetes were reported by the following researchers.

Edward O'Brien, MD, FRCPC, (Libin Cardiovascular Institute of Alberta, University of Calgary, Alberta, Canada) Edward O'Brien proposed that Hsp27 has a role in maintaining vascular health. The mechanisms by which Hsp27 exerts these protective effects by interacting with cells of the cardiovascular system is not completely understood. Dr. O'Brien described how Hsp27 activates NF- κ B signaling in macrophages resulting in altered expression of both pro-inflammatory and anti-inflammatory factors that may play important roles in atherogenesis and lesion progression/remodeling. Hence, mechanisms of Hsp27 release and signaling, and the description of putative cell surface receptors were described.

Juan Claudio Benech (Laboratory of Cell Signaling and Nanobiology, Instituto de Investigaciones Biológicas "Clemente Estable", Montevideo, Uruguay) Oxidative stress resulting from increased production of ROS (or their inadequate removal) plays a key role in the pathogenesis of late diabetic complications. This conference demonstrated an interesting multidisciplinary research approach where the use of nanotechnologies proved useful for the detection of cellular

stress at the physiological level. For instance, this talk proposed a model to study diabetes by the use of atomic force microscopy (AFM) nanoindentation and also studies that show the restorative effects of Resverastrol. The results presented suggest that live cardiomyocyte material properties were affected by diabetes, causing as a final result stiffer cells. Diabetic effects on cardiomyocyte elasticity can be partially prevented with resveratrol treatment.

María Bausero (Laboratory of Cell Signaling and Nanobiology, Instituto de Investigaciones Biológicas “Clemente Estable”, Montevideo, Uruguay) María Bausero presented preliminary data on the effects that treatment with superparamagnetic iron oxide nanoparticles (SPIONS) have in cell stress. As an alternative way of specifically treating tumor cells with siRNA-Hsp27 linked SPIONS, they proposed that although nanoparticles have been proved nontoxic, little is known of further unwanted molecular stress induction that may have an impact on the safety of biomedical applications and pharmaceuticals. HSF-1 induction, cell rigidity, LPS content, induction of cytokines, among others, are some of the parameters currently under investigation. We are developing a quality control system for nanotechnologies of oncotherapeutics.

Concluding remarks

The diversity of topics and the innovative fields presented by the invited speakers and the quality of the posters provided the strongest evidence of the relevance of the field of heat shock proteins and chaperones in Latin America. We celebrate the contributions to this agenda made by both the CSSI and the team of Latin American researchers. Times are rapidly changing in the research politics of several Latin American countries concerned with progress and development. The heat shock proteins and chaperones community of scientists is an increasingly important part of it.

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