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Abstract/Resumen: Bovine anaplasmosis is caused by the intraerythrocytic rickettsia Anaplasma marginale. It is an arthropod-borne hemolytic disease characterized by high lethality in adults. The disease is expanding and it is a threat to cattle industry. Live vaccine based on A. centrale, a less pathogenic species, is used to prevent acute anaplasmosis. Competition ELISA (cELISA) is used to assess rodeo epidemiological status through the detection of anti-MSP5 antibodies. A. marginale MSP5 (MSP5m) and A. centrale MSP5 (MSP5c) have 91 % of identity and cELISA does not differentiate A. marginale-infected from A. centrale-vaccinated cattle. In this work, a cELISA (cELISAm) was developed to detected specific A. marginale antibodies. cELISAm is based in the antigen A. marginale MSP5 and the Am6 monoclonal antibody. Am6 recognized an A. marginale specific epitope, absent in A. centrale. ELISA plates were coated with MSP5m (1 µg/well) overnight at 4 ºC. After blocked, the plates were incubated with serum samples diluted 1:2 in PBST/10 % fat-free dried milk containing 35 µg/ml MSP5c. Then, Am6 1/2500 and anti-mouse IgG peroxidase conjugate 1/3000 in PBS were added consecutively. The reaction was revealed with ABTS/H_2O_2. Results were expressed as inhibition percentage (%I). A total of 744 serum samples, previously classified by cELISA and nPCR, were analyzed by cELISAm: 497 negative, 185 A. marginale positive and 62 A. centrale positive serum samples. Sensitivity and specificity of the cELISAm were 97 and 95 %, respectively; with a cut off >18 %I. The 65 % of A. centrale-vaccinated cattle were not detected by cELISAm. Preliminary results showed that the cELISAm developed, allows us to detect with high sensitivity A. marginale-infected cattle decreasing the cross reaction for A. centrale. The identification of the specific epitope recognized for Am6 and the use of the polypeptide of this epitope in a cELISA with Am6, could enhance the performance of this specific cELISAm in the future.

0398 - HUMORAL IMMUNITY CONFERRED BY FOUR RECOMBINANT PROTEINS FROM NEOSPORA CANINUM ADJUVANTED WITH LIPOSOMES AND CPG-ODN IN CATTLE.

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Abstract/Resumen: The apicomplexan protozoan Neospora caninum is a major cause of abortion in cattle with the consequent severe economic losses to production. It persists for life in tissue cysts with periodic reactivations. The present study was designed to evaluate the immunity generated by the recombinant proteins NcMIC1, NcMIC3 (micronemes), NcSRS2 (tachyzoites surface) and NcGRA7 (dense granules), adjuvanted with liposomes (Lip) and CpG oligodeoxynucleotides (CpG-ODN) against N. caninum infection and persistence. Recombinant His-tagged proteins expressed in Escherichia coli were purified by Ni-NTA affinity chromatography. Eighteen 3-year-old steers were divided in three groups (G) of six steers each. GA animals were inoculated with 100 µg of each recombinant protein and Lip+CpG-ODN. GB received Lip+CpG-ODN adjuvant without antigen and GC received sterile phosphate buffered saline (PBS). Steers were inoculated twice (days 0 and 21) and were challenged with 1 million tachyzoites of NC-1 strain at day 56 after the first dose (afd). Serum samples were collected weekly until day 102 afd. Indirect ELISA (iELISA) based on the four recombinant proteins (iELISAr) or N. caninum tachyzoites lysate (iELISAtach) were used to measure specific antibodies induced by the proteins. An increase in the level of antibodies against the four proteins were detected by iELISAr at day at day 14 afd and after challenge at day 75 afd in steers from GA compared with those from GB and GC ($p<0.001$). The iELISAtach detected antibodies against N. caninum 12 days after challenge. The antibody titer was higher in steers from GA than in steers from GB and GC ($p<0.001$). In conclusion, NcMIC1, NcMIC3, NcSRS2 and NcGRA7 recombinant proteins were immunogenic. The presence of tissue cyst in the brain of all cattle after slaughter is under analyzes.

0762 - ABILITY OF CATIONIC AND NEUTRAL HYDROGELS BASED ON N-ISOPROPYLACRYLAMIDE TO BIND EQUINE SPERMATOZOA.

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Abstract/Resumen: PNIPAm (poli(N-isopropylacrylamide) based hydrogels are biocompatible materials extensively used in biomedicine. The aim of this study was to evaluate the ability of PNIPAm hydrogels to attach stallion spermatozoa. PNIPAm was co-polymerized with cationic 3-(acrylamido propyl) trimethylammonium chloride (APTA) or neutral N-[Tris (hydroxymethyl) methyl] acrylamide (HMA) monomers. Studied copolymers contained 5 % APTA, 10 % APTA, 15 % APTA, 20 % HMA and 20 % HMA semi-interpenetrated with hyaluronic acid (20 % HMA-HA, 1 mg/mL). Each hydrogel was placed in a culture dish containing TALP medium and fresh stallion sperm suspension was added (1×10^6). The percentage of sperm attached to the surfaces was determined from the difference between the number of sperm initially added into the culture dish and the recovered non-bound cells after incubation (30 min at 37 °C in 5 % CO_2). Sperm that were exposed to -20 °C for 60 min to render them non-viable were incubated with 15 % APTA and 20 % HMA hydrogels-containing dishes, which served as negative controls. Data was analyzed by one way ANOVA; a $p<0.05$ was considered to be significant. Higher percentage of spermatozoa was attached to the surface with APTA compared to the HMA hydrogels ($p<0.05$). The percentage of sperm bound to APTA hydrogels did not differ among the cationic monomer concentrations (5 % APTA: 94.0 ± 4.0 %; 10 % APTA: 79.6 ± 13.2 %; 15 % APTA: 84.7 ± 14.7 %; $p>0.05$). The semi-interpenetration of 20 %HMA hydrogels with HA did not increase the percentage of spermatozoa bound to the surface (20 % HMA: 43.4 ± 7.9 %; 20 % HMA-HA: 50.5 ± 4.9 %; $p>0.05$). Few non-viable spermatozoa were observed attached to hydrogels (20 % HMA: 16.1 ± 8.3 %; 15 % APTA: 15.3 ± 4.8 %). In conclusion, cationic PNIPAm hydrogels could be an efficient support and binding substrate to adhere viable equine sperm, since stallion spermatozoa attaches more efficiently on them. It is expected that this strategy will allow the development of a technique for sperm selection, which isolates the cell subpopulation with high structural and functional quality characteristics, improving the efficiency of assisted reproduction techniques in horses.

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Biología celular y molecular de procesos fisiológicos y patológicos / Biology I

Chairs: Alejandra Erlejman | Gabriela Lombardi

0057 - 20-HYDROXYEICOSATETRAENOIC ACID (20-HETE) PROMOTES A MALIGNANT PHENOTYPE IN HUMAN CASTRATION-RESISTANT PROSTATE

CANCER CELLS THROUGH STIMULATION OF THE G PROTEIN-COUPLED RECEPTOR GPR75.

Sofia CARDENAS (1) | Cecilia COLOMBERO(1) | Laura PANELO(2) | Rambabu DAKARAPU(3) | John FALCK(3) | Mónica COSTAS(2) | Susana NOWICKI(1)

CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE)-CONICET (1); INSTITUTO DE INVESTIGACIONES MÉDICAS ALFREDO LANARI - UNIVERSIDAD DE BUENOS AIRES (2); UT SOUTHWESTERN MEDICAL CENTER (3)

Abstract/Resumen: 20-Hydroxyeicosatetraenoic acid (20-HETE), the product of 20-hydroxylation of arachidonic acid by cytochrome P450 isoforms (CYP4F2 and CYP4A11), has a role in the oncogenesis of several human tumors. Recently, the GPR75 receptor has been identified as the target for 20-HETE. We have shown that androgen independent prostate cancer cells (PC-3) express GPR75. The aim of this study was to assess in vitro if 20-HETE/GPR75 modify the metastatic features of PC-3 cells. Cells were incubated with 20-HETE or its stable analog 5,14-HEDGE (both 0.1 nM) in the presence or absence of two different antagonists of the 20-HETE receptor, AAA or 19-HEDE (both 5 or 10 uM). The following assays were performed: e-cadherin and vimentin protein expression (epithelial-mesenchymal transition), zymography (release of matrix metalloproteinase-2 (MMP-2)), immunofluorescence and p-FAK (changes of cytoskeleton), scratch wound healing (migration), and soft agar colony formation (anchorage-independent growth). Results were analyzed using one-way ANOVA followed by Dunnet's. 20-HETE (24 h) increased by 150 % the expression of vimentin ($p<0.0001$, n= 3) and diminished by 40 % the expression of e-cadherin ($p<0.0001$, n= 3), whereas these effects were reversed by AAA ($p<0.0001$ and $p<0.05$, respectively). 20-HETE increased by 52 % the release of MMP-2 ($p<0.05$, n= 3), and this was also inhibited by AAA ($p<0.001$). AAA disorganized the actin filaments throughout PC-3 cells, while tubulin filaments remained unchanged. Also, 20-HETE increased by 89 % FAK phosphorylation (Y397) ($p<0.0001$, n= 3). 20-HETE increased by 147 % cell migration rate ($p<0.0001$, n= 3) and this effect was reverted by both antagonists, AAA or 19-HEDE ($p<0.05$ and $p<0.0001$, respectively), or by knockdown of GPR75 ($p<0.0001$). Finally, 5,14-HEDGE (21 days) formed twice the number of colonies vs. control ($p<0.05$, n= 2) and this was abolished by AAA ($p<0.05$). These results strongly suggest a role for GPR75 in 20-HETE-mediated metastatic features in PC-3 cells.

0072 - INTRACELLULAR CL- MODULATION OF IL-1ß SECRETION AND THE NLRP3 INFLAMMASOME EXPRESSION/ACTIVITY REQUIRE SGK1

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Abstract/Resumen: The impairment of the CFTR activity induces intracellular chloride $[Cl^-]_i$ accumulation and consequently, as a second messenger, stimulates the secretion of interleukin-1ß (IL-1ß). We have previously described that this secretion starts an autocrine positive feedback loop. Moreover, the expression of two subunits of the inflammasome complex: NLR family pyrin domain containing 3 (NLRP3) and caspase-1 (CASP1), that are involved in the IL-1ß maturation, are indirectly modulated by the $[Cl^-]_i$. On the other hand, cellular and mitochondrial ROS (reactive oxygen species) also are regulated by $[Cl^-]_i$. Recently, other authors found that differences in $[Cl^-]_i$ modulates SGK1 (serum-glucocorticoid kinase 1) phosphorylation and subsequently regulates NF-kB activation in airway epithelial cells. Therefore, we decided to study the effects of SGK1 on IL-1ß expression at different $[Cl^-]_i$. In this study we used IB3-1 cells (a bronchial cell line derived from a cystic fibrosis patient with a DF508/W1282X CFTR genotype) and Caco-2 cells (transfected with CFTR-shRNA). The cells were incubated for 1 h at 5 or 75 mM Cl^-, in presence of ionophores tributyltin (10 µM) and nigericin (5 µM) to equilibrate $[Cl^-]_e$ and $[Cl^-]_i$. To explore if SGK1 was also involved in the IL-1ß response to $[Cl^-]_i$, we used the SGK1 inhibitor GSK650394 at 0, 0.1, 1 and 10 µM. After, we determine IL-1ß expression by quantitative real-time RT-PCR and ELISA quantification in culture media. To analyze the ROS response, we determined DCF fluorescence and MitoSOX fluorescence by microplate reader and/or flow cytometry. The results showed that SGK1 inhibitor diminished the response of IL-1ß mRNA to changes in the $[Cl^-]_i$ from 5 to 75 mM; GSK650394, at 10 µM, completely abrogated the IL-1ß mRNA response to Cl^- 75 mM ($p<0.05$, n= 3). Similar results were obtained on the secreted IL-1ß. On the other hand, SGK1 inhibitor, significatively reduced both, cellular and mitochondrial ROS levels at 75 mM Cl^- ($p<0.05$, n =3), suggesting that both the IL-1ß loop and the ROS response to Cl- were blocked by GSK650394. Similar results were found in Caco-2 with CFTR-shRNA. The results suggest that Cl^- effects are indirectly mediated by SGK1, which under Cl^- modulation stimulates the secretion of mature IL-1ß, in turn responsible for the observed upregulation of ROS and CASP1, NLRP3, and IL-1ß itself. The exact point of SGK1 action is still unknown.

0074 - INTRACELLULAR SIGNALING PATHWAYS TRIGGERED BY THE STIMULATION OF THE G-COUPLED PROTEIN RECEPTOR GPR75 BY 20-HYDROXYEICOSATETRAENOIC ACID (20-HETE) IN ANDROGEN INDEPENDENT PROSTATE CANCER CELLS.

Sofia CARDENAS (1) | Cecilia COLOMBERO(1) | Laura PANELO(2) | Rambabu DAKARAPU(3) | John FALCK(3) | Mónica COSTAS(2) | Susana NOWICKI(1)

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Abstract/Resumen: 20-HETE, the product of 20-hydroxylation of arachidonic acid by cytochrome P450 isoforms (CYP4F2 and CYP4A11), has a role in the oncogenesis of several human tumors. Recently, the GPR75 receptor has been identified as the target for 20-HETE. We have shown that androgen independent prostate cancer cells (PC-3) express GPR75. The aim of this study was to identify intracellular signaling molecules activated upon GPR75 stimulation by 20-HETE in PC-3 cells. Cells were incubated with 20-HETE (0.1 nM) in the presence or absence of the antagonist of the 20-HETE receptor, AAA (5 or 10 µM). Protein expression of the inducible focal adhesion protein Hydrogen Peroxide Inducible Clone-5 (HIC-5), the phosphorylated and total form of NF-kB, AKT, p38 MAP-Kinase (p38) and EGFR were assessed by Western blot. Intracellular localization of p-AKT, NF-kB and PKCa were determined by immunofluorescence and subcellular fractionation. Results were analyzed using one-way ANOVA followed by Dunnet's. Incubation with 20-HETE (2 h) increased the phosphorylation of EGFR, NF-kB and AKT by 146, 172 and 219 %, respectively (vs. control, $p<0.01$ for NF-kB, and $p<0.001$ for EGFR and AKT, n= 3), and this was inhibited by AAA (vs. 20-HETE alone, $p<0.05$ for NF-kB, $p<0.01$ for AKT and $p<0.001$ for EGFR). AAA alone increased p-38 phosphorylation by 248 % ($p<0.001$ vs. control, n= 3). 20-HETE (1 h) induced the translocation of p-AKT to the nuclei ($p<0.001$, n= 3) and promoted the redistribution of PKCa out of the nuclei ($p<0.05$, n= 3) to the plasma membrane ($p<0.001$). Both effects were inhibited by AAA (vs. 20-HETE, $p<0.01$ for AKT and $p<0.05$ for PKCa). AAA alone reduced the nuclear signal of p-AKT and NF-kB, usually activated in tumoral cells ($p<0.001$ for both, n= 3). Additionally, 20-HETE (12 h) increased by 150 % the protein expression of Hic-5 ($p<0.0001$, n= 5) and this was abolished by AAA ($p<0.001$). Our results show that 20-HETE modulates signaling pathways known to be deregulated in malignant cells through the GPR75-axis.