the process of tissue repair.

712. (719) **CURCUMIN LOADED POLY LACTIC-CO-GLYCOLIC ACID NANOPARTICLES AS AN ANTIVIRAL FORMULATION AGAINST ZIKA VIRUS**

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Antiviral drugs in current use target only viral proteins; are, generally, specific for each type of virus and frequently induce the rapid emergence of resistant mutants, a particularly serious problem for RNA virus therapy due to its high mutation rate. The development of drugs that affect host factors necessary for viral multiplication that involve cellular pathways has proven to be attractive for chemotherapeutic intervention against viruses of the same genus, family and even unrelated, since it is not expected that the individual viral mutations compensate for the loss of a required factor of the host. One mechanism to control rapidly replicating viruses is to manipulate the levels of cellular nucleoside triphosphate pools. Inosine-5′-monophosphate dehydrogenase (IMPDH) is an enzyme involved in the de novo synthesis of guanine nucleotides. Inhibitors of IMPDH such as ribavirin, mycophenolic acid and merimepodib have shown activities against Chikungunya, Junín, Lassa, several flavivirus members and Ebola viruses. Other authors found that several polyphenols showed inhibitory effect on IMPDH, in particular, curcumin was the more active and exerted a competitive and uncompetitive actions to suppress the IMPDH activity. In the present work, the in vitro antiviral effect of curcumin against Zika virus was studied. Firstly, the toxicity was determined for 48 h in monkey Vero cells by de MTT assay. In order to minimize cytotoxicity and maximize the curcumin cell delivery, curcumin loaded poly lactic-co-glycolic acid (PLGA) nanoparticles were synthetized. No cytotoxic effects were observed until 150 μM. Through a viral yield inhibition assay, a dose-dependent viral replication inhibition was observed in a 0-100 μM curcumin concentration range. Our previous reports with other inhibitors and RNA virus, plus these results show that IMPDH is a promissory antiviral target to control pathogenic viral infections and curcumin loaded PLGA nanoparticles may be a good candidate drug formulation.

713. (752) **PATHOGEN-SPECIFIC T CELLS FROM PATIENTS WITH INFLAMMATORY BOWEL DISEASES CAN BE MODULATED BY PROBIOTICS FROM KEFIR**

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Kefir is a fermented milk with health-promoting properties. Lamina propria T cells (LPTC) play a central role in the pathogenesis of Inflammatory Bowel Diseases (IBD). These cells contribute to mucosal inflammation by secreting pro-inflammatory cytokines and being resistant to apoptosis. We aimed to modulate the proliferation and the pro-inflammatory response of pathogen-specific LPTC from IBD patients using microorganisms from kefir.
Colonic biopsies from IBD patients were incubated with probiotics from kefir and cytokines in supernatants were evaluated by ELISA afterwards. LPTC were isolated from biopsies (N=23; 7 from Crohn's Disease patients-CD and 15 from ulcerative colitis-UC patients) by collagenolytic digestion. In order to expand pathogen-specific T lymphocytes, cells were cultured with enteroadhesive (EA) *Escherichia coli* extract and IL-2 for 10 days. Thereafter, LPTC were incubated with anti-CD3/anti-CD28 and with microorganisms from kefir (*Enterococcus durans* and *Lactobacillus kefiri*), their conditioned media or 10 μM lactate for 96 hs. LPTCs proliferation (CFSE) and TNF-α, IFN-γ, IL-6, IL-10 and IL-13 cytokine secretion were evaluated (ELISA).
We found that *E. durans* and *L. kefiri* diminished the pro-inflammatory cytokine production by inflamed tissues (IL-6: 1800±670 and 576±3 vs 6900±2000 pg/ml basal production, respectively; IL-8: 38000±11000 and 32000±7300 vs 80000±27000 pg/ml basal production, respectively). EA E. coli specific LPTC lines were developed from all patients. Cellular proliferation of activated CD4+/CFSE-/PI- LPTC decreased significantly with *L. kefiri* , *E. durans* and lactate ($P<0.01$, N=15), but not with conditioned media. Moreover, TNF-α ($P<0.05$, N=10), IFN-γ ($P<0.05$, N=11) and IL-6 ($P<0.05$, N=8) secretion decreased with the presence of probiotics, their supernatants or lactate. No significant differences were observed for IL-10 and IL-13.
Our results showed that probiotic strains from kefir and their metabolites modulated pathogen-specific activated T cells from IBD patients. These results could contribute to future therapeutic approaches for IBD.

714. (767) **EVALUATION OF TRITRICHOMONAS FOETUS INFECTION CLEARANCE IN HEIFERS IMMUNIZED WITH A SINGLE INTRAVAGINAL DOSE OF FORMALDEHYDE FIXED CELLS**

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Tritrichomonas foetus is a flagellated parasitic of the urogenital tract of cattle that causes a sexually transmitted disease characterized by embryonic and fetal losses. Vaccines against *T. foetus* have shown to reduce time of infection after natural or experimental challenge. Usually, vaccine strategies are three systemic doses reinforced by a vaginal booster. The object of this study was to assess protection against *T. foetus* infection conferred by fixed hole cells given in a single vaginal instillation. *Aberdeen Angus* virgin heiffers were randomly assorted into 3 groups of 12 individuals to receive placebo, fixed cells with formalin and fixed cells with freshly prepared formaldehyde solution, challenged six weeks later with 106T. foetus motile cells. The median clearance rates among control heifers was 93.75 days while in animals immunized with formaldehyde fixed cells was 45 days. A single vaginal dose of cells fixed with fresh formaldehyde solution showed a rate of infection decay per unit of time of 2.54 (CI 95%=1.07;6.01). Further *in vivo* studies are necessary to confirm our observations and potentially change the current thinking about *trichomonas* infection/protection.

715. (768) **IMMUNOMODULATORY PROPERTIES OF PROSTAGLANDIN E2 ON NEUTROPHILS DURING HUMAN TUBERCULOSIS INFECTION.**

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During *Mycobacterium tuberculosis* (*Mtb*) infection, neutrophils are among the first cells that migrate to the infectious focus and represent the most abundant cell population harboring *Mtb* in samples from patients with active tuberculosis (TB). Neutrophilic infiltration and exuberant inflammation represent characteristic features of severe TB, but neutrophil biology during TB remains poorly understood. Prostaglandin E2 (PGE2), an active lipid compound, is a key mediator of immunopathology in chronic infections . Manipulation of PGE2 levels was proposed as an approach for countering the Type

I IFN signature of TB patients, but very limited information is available regarding this pathway in TB patients. The aim of this work was to investigate the role of PGE2 on the modulation of human neutrophil immune responses against *Mtb*. We detected a significantly higher number of neutrophils in patients with positive Acid Fast Bacilli (AFB) in sputum as compare to negative AFB TB patients ($p<0.01$). Besides, stimulation of neutrophils from healthy donors (HD) with an *Mtb* lysate (*Mtb*-Ag, 10 µg/ml) significantly enhanced CD11b expression, a marker of neutrophil activation. Interestingly, CD11b levels were markedly reduced by effect of PGE2 (2 µg/ml). Furthermore, for the first time to our knowledge, we observed that neutrophils from HD and TB patients express SLAMF1, a costimulatory molecule recently described as a microbial sensor. Also, *Mtb*-Ag stimulation significantly increased the percentage of SLAMF1+ neutrophils ($p<0.05$), but PGE2 treatment diminished SLAMF1 levels ($p<0.05$). Moreover, neutrophils stimulated with *Mtb*-Ag augmented LC3B-II levels as compared to non-stimulated cells. Importantly, PGE2 treatment significantly increased autophagy flux in human neutrophils stimulated with *Mtb*-Ag ($p<0.05$). Therefore, autophagy, a process involved in the defense against Mtb, might prevent excessive inflammation and tissue damage. Taken together, our findings extend the information about the mechanisms mediated by PGE2 that operate during the human immune response to *Mtb*.

716. (782) **MURINE ANTIGEN-PRESENTING CELLS INTERNALIZE BINARY ETHYLENIMIDE INACTIVATED FOOT-AND-MOUTH DISEASE VIRUS AND RELEASE EXTRACELLULAR VESICLES EXPRESSING VIRUS PROTEINS.**

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Extracellular vesicles (EVs) secreted by antigen-presenting cells (APC) play a crucial role in carrying and presenting major histocompatibility-peptide complexes, but can also spread pathogen and host-derived molecules during infections. Foot-and-mouth disease virus (FMDV) is a highly contagious disease of livestock worldwide and is economically important. The main strategy for the control is vaccination with virus chemically inactivated with binary ethylenimide (BEI-FMDV). The protection achieved is good, however, requires regular re-inoculations to produce sustained immunity over time.
In the present work we aimed to study whether APC differentiated from bone marrow cells are able to internalize BEI-FMDV and release extracellular vesicles. For this purpose, APC were differentiated from murine bone marrow cells with GM-CSF. On day 8, more than 85% of the cells expressed CD11c and MHC-II molecules. By flow cytometry we observed that 30% of APC internalized purified BEI-inactivated virions labelled with FITC (BEI-FMDV-FITC) after incubation for 60 min at 37°C with APC. EVs were isolated by centrifugation, ultrafiltration and ultracentrifugation from supernatant of APC pulsed with BEI-FMDV or LPS for 18 h and characterized by flow cytometry. EVs derived from APC pulsed whit FMDV (1, 5 and 10 µg/ml) demonstrated strong staining for the EVs marker CD9 (45%, 93%, 81%, respectively) and CD81 (9 %, 58% and 40 %). APC molecules such as MHC-II (>90%) and CD86 (>40%) were also expressed. Remarkably, FMDV antigens were expressed on EVs derived from APC incubated with 5 µg/ml FMVD.
Our results show that inactivated FMVD can be internalized by APC and these cells release EVs expressing FMDV molecules and APC cells markers.
The knowledge derived from this work will serve to deepen the knowledge of the interrelation between the FMDV and the immune system that will serve for the rational design of vaccines.

717. (784) **REDUCED TRP-IDO-AHR AXIS ACTIVITY IS ASSOCIATED WITH HIGHER LEVELS OF INFLAMMATORY CYTOKINES IN PATIENTS WITH SEVERE CHRONIC CHAGAS CARDIOMYOPATHY**

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After acute infection with *T. cruzi* approximately 30% of infected individuals develop Chronic Chagas cardiomyopathy (CCC) while the rest remain asymptomatic (Asy). Although the mechanisms underlying the differential progression to CCC are still not fully understood, CCC display a more intense inflammatory response than Asy patients, who appear to have a more regulated immune response. We have reported that the indoleamine 2,3 dioxygenase-tryptophan metabolites-aryl hydrocarbon receptor (IDO-Trp-AhR) axis is associated with both the development of a strong Th1 response able to control parasite replication and its regulation by inducing, depending on the levels of AhR activation, Treg or IL-10+ producing cells. AhR can be activated by several ligands many of them being derivatives of Trp, such as L-kynurenine (Kyn) generated by IDO activity. To determine whether IDO-Trp-AhR axis is associated with CCC development, we analyzed the levels of IL-6, TNF, Trp metabolites and AhR agonists in serum samples from healthy controls, CCC and Asy patients by using ELISA, HPLC, targeted LC-MS and AhR agonistic activity. CCC patients were subclassified as Mild (altered ECG without congestive cardiac failure) or Severe (altered ECG, congestive cardiac failure and other alterations). By using a luciferase plasmid reporter assay we detected a decreased global AhR agonistic activity in infected patient sera as compared to healthy controls ($p<0,0001$), with Mild patient's sera showing lower levels than Asy and Severe patients ($p<0.05$). Moreover, infected patients showed increased levels of circulating Kyn (HPLC, $p=0.01$; LC-MS, $p<0.0001$), IL-6 and TNF compared to healthy controls. In addition, Severe patients' sera presented lower levels of Kyn ($p<0.05$) and higher levels of Trp, IL-6 and TNF than those observed in Asy patients ($p<0.05$). Our results support an association between higher levels of inflammatory cytokines and reduced Trp-IDO-AhR axis activity in Severe patients. Analysis of targeted metabolomic data are underway in our laboratory.

718. (796) **MECHANISM OF THE EFFECTS OF 5,5-DIMETHYL 1-PYRROLINE N-OXIDE IN A MOUSE MODEL OF ACUTE RESPIRATORY DISTRESS SYNDROME**

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The nitrones spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO) was developed for the study of free radicals. Later it was used to visualize protein- and DNA-centered radicals in cells, tissues and whole animals in animal models of diseases. Recently we found that DMPO blocks lipopolysaccharide (LPS)-triggered signaling in macrophages primed with LPS, traps protein- and DNA-centered radicals, and also decreases systemic inflammation and death in mice exposed to an overdose of LPS. Herein we aimed at determining the mechanism by which DMPO can protect the lung in a mouse model of acute-distress respiratory syndrome (ADRS) triggered by bacterial LPS. We hypothesize that these protective effects of DMPO can either be due to blocking quimiotaxis/homing and activation of neutrophils, myeloperoxidase (MPO) activity or other enzyme sources of reactive biochemical species o by trapping protein radicals and thus reducing their decay to end-oxidation products. We used male C57 mice (7 weeks-old, 9/group) and exposed them, under light anesthesia, to oropharyngeal aspiration of 50 ul of either vehicle (PBS) or 1 ug/ul LPS. Another group of animals (9/group) were pre-treated 30 min before LPS challenge with vehicle or vehicle containing 50 mM DMPO (2.5 nmol DMPO/mice). We found that LPS treatment increased ICAM-1 and iNOS expression, neutrophil in lung parenchyma (NIMP-14+ cells); and markers of tissue damage in BALF (LDH leakage); MPO protein and activity in lung parenchyma and BALF; and chlorotyrosine, nitrotyrosine and carbonyls in the lung parenchyma. These effects were prevented by pretreatment with