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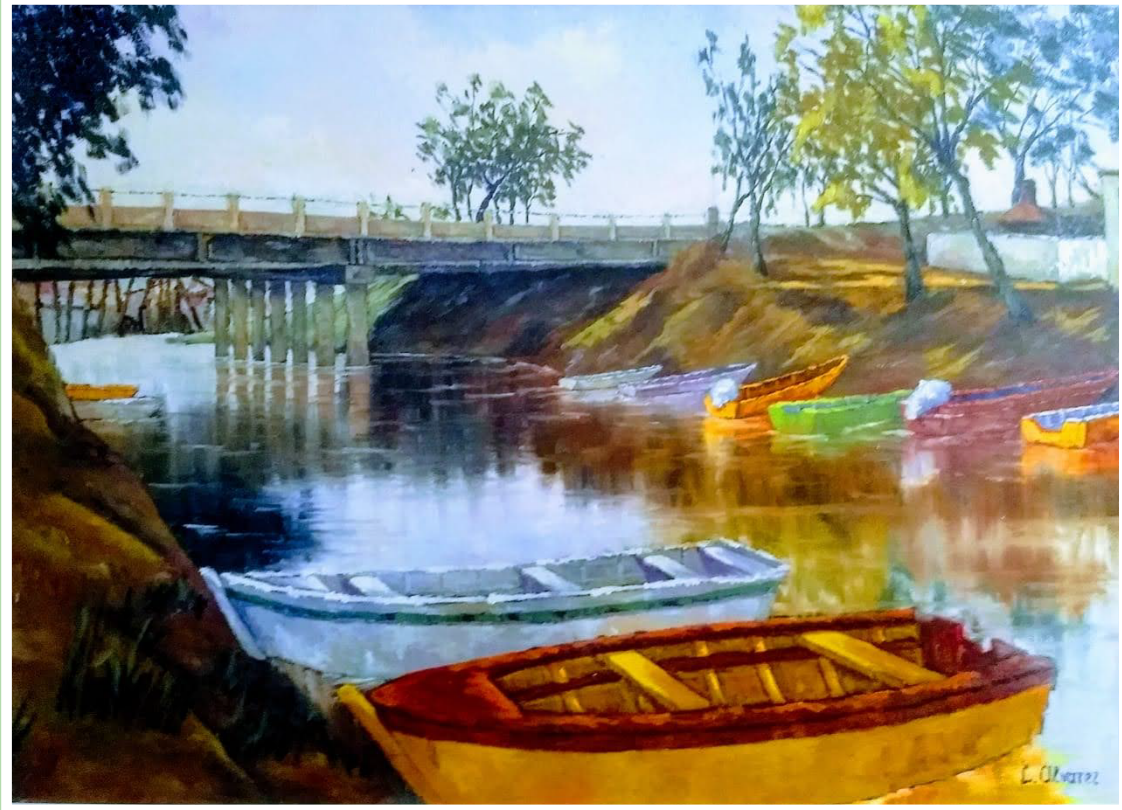

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LXV REUNIÓN ANUAL DE LA
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10-13 de noviembre de 2020

EDITORES RESPONSABLES
María Cristina Carrillo
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conditioned media (CM) of human endometrial stromal cell line decidualized with MPA+dbcAMP for 8 days (Dec-CM) or not (Non-dec CM) were collected. Then, isolated monocytes from peripheral blood mononuclear cells of healthy women were cultured with rhGM-CSF+rhIL-4 for 5 days in the absence/presence of CM.
Whereas both Non-dec and Dec-CM-cultures showed increased IL-10 secretion by monocyte-derived cells, only Dec was able to induce a higher expression of the characteristic DC-10 tolerogenic markers HLA-G and ILT2/CD85j ($p<0.05$, Friedman test). Then, Dec and Non-dec-CM-treated cells were co-cultured with allogeneic lymphocytes for 5 days. On the last day, we observed an anti-inflammatory microenvironment in Dec-CM-cultures, characterized by a higher IL-10:IFN-γ ratio production and a decreased $CD4^+CD25^+$ subset ($p<0.05$, Friedman test). Interestingly, while both co-cultures showed a higher frequency of $CD4^{neg}HLA\text{-}G^+$ cells, only Dec-CM-treated cells were able to induce $CD4^+HLA\text{-}G^+$ regulatory T cell subset ($p<0.05$, Friedman test). The present results suggest that decidualization process promotes the differentiation of DC-10 subset able to induce $HLA\text{-}G^+$ T cells that might play an immunoregulatory role in embryo implantation.

392. (442) **ANTIGEN PRESENTING CELLS PULSED WITH INACTIVATED FMDV REALEASE EXTRACELLULAR VESICLES WITH THE ABILITY TO ACTIVATE BOTH SPECIFIC T AND B-CELLS IN VITRO.**

Menay F [1], Cocozza F [2], Gravisaco MJ [3], Elisei A [1], Re J [1], Sampedro P [4], Ferella A [1] y Mongini C [1].
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Foot-and-mouth disease (FMD) is a highly contagious disease of livestock worldwide and is economically important. The main strategy for the control is vaccination with FMD-Virus (FMDV) chemically inactivated with binary ethylenimide (FMDVi). In FMDV infection and in vaccination, the B cell response plays a major role by providing neutralizing/protective antibody in both animal models and natural hosts. Extracellular vesicles (EVs) are nanovesicles involved in cell–cell communication. EVs secreted by antigen-presenting cells (APC) participate in the activation of B and T cells through the presentation of native antigen membrane associated (to B cells) or by transferring MHC-peptide complexes (to T cells) and even complete antigen from DCs. In our previous work we demonstrated that murine APC cells can internalize FMDVi and release EVs expressing APC markers and high level of viral proteins during the first 24 h. In the present work we aimed to evaluate the immune properties of these EVs in the generation of B and T cell response against FMDV. We demonstrated that EVs-FMDVi induced specific in vitro proliferation in vivo sensitized splenocytes with FMDVi, EVs-FMDVi induced specific B cell (16.05% ± 0.61 $p<0.001$) and T cell proliferation (8.5% ± 0.81 $p<0.01$) when compared to unstimulated sensitized splenocytes (9.66% ± 0.17 and 5.70% ± 0.15, respectively) detected by CFSE dilution.
Our results revealed that EVs FMDVi could present part of FMDV proteins in native conformation or partially processed. These peptides can be recognized by the BCR and stimulate specific B cells response against viral infection. In addition, EVs FMDVi activate direct or indirectly a T cell response that could collaborate in B cell activation.
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.

393. (500) **CLUSTERIN EXPRESSION PROMOTES T CELL PRIMING BY DENDRITIC CELLS**

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Clusterin is a multifunctional glycoprotein present in almost all tissues and body fluids. It is involved in a number of physiological and pathological processes including apoptosis, protein homeostasis, Alzheimer´s disease and cancer. Although clusterin expression by myeloid cells has been reported, its influences on dendritic cell (DC) function have not been analyzed. Here we show that clusterin expression by DCs plays a role in their ability to initiate the adaptive immune response.
As we did before using monocyte derived DCs, we analyzed the expression of clusterin by BDCA+ blood DCs. We found that clusterin production was induced after LPS stimulation (mean 1,61 ng/ml unstimulated vs 6,86 ng/ml stimulated, n=4, $p<0,05$).To look into the role of clusterin on DC function we performed a knockdown (KD) strategy using clusterin shRNA carrying lentiviruses and a scramble (SCR) construction as a control. In a previous report we showed that silencing of clusterin expression (CLU-KD) resulted in an increased cell death of DCs upon LPS stimulation. We now analyzed the function of CLU-KD-DCs in response to LPS stimulation. Control and CLU-KD DCs were stimulated with LPS for 24hs, cells were collected and analyzed by FACS and supernatants were harvested and cytokine secretion was measured by ELISA. We found that phenotype maturation markers (DR, CD80, CD86, CD40 and PDL1) and cytokine secretion (IL-1, IL-6, TNF, IL-12 and IL-10) were not modified after clusterin silencing (not shown). We next studied the role of clusterin in antigen presentation culturing control and CLU-KD DCs with allogenic CD4+ T cells. Interestingly, CLU-KD dendritic cell ability to expand CD4 was found to be strongly diminished (mean 33,87 %CFSE- CLU-KD vs 19,15 CLU-KD LPS, n=6, Paired T test $p<0.01$).
These observations suggest that clusterin might play a role in the control of the adaptive immune response.

394. (504) **HUMORAL AND CIRCULATING FOLLICULAR HELPER T CELLS RESPONSES IN HOSPITALIZED INFANTS WITH COVID-19**

Sananez I[1], Raiden S[2], Algieri S[3], Grisolia N[2], DiFilippo D[4], De Carli N[5], Cairoli H[2], Chiolo MJ[2], Di Lallia S[2], Lattner J[6], Coll P[7], Rolón MJ[7], Bruera MJ[3], Sarli M[3], Uranga M[8], Seery V[1], Holgado P[1], Ferrero F[2], Geffner J[1], Arruvito L[1].
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Background: Marked progress is achieved in understanding the physiopathology of COVID-19 pandemic. However, CD4+ TFH cell subset, which is critical for eliciting neutralizing antibodies, is poorly understood during pediatric COVID-19.
Aims: To characterize the circulating TFH (cTFH) cell subset and to determine SARS-CoV-2 IgM and IgG antibody response in children hospitalized with acute mild and severe (pediatric multisystem inflammatory syndrome, PIMS) COVID-19, compared to pre-pandemic controls.
*Methods:*Sera (n=337) and PBMCs (n=41) from hospitalized COVID-19 children (age 1-14yr.) during different phases after disease onset and healthy donors (n=15, age 1-10 yr., HD) were collected to evaluate:
1.Memory cTFH subset (CD3+CD4+CD45RA-CXCR5+) and cTFH profiles (cTFH1 –non-efficient helper associated with a suboptimal antibody production-, cTFH2, and cTFH17, –efficient helpers) by

flow cytometry during 1st week of COVID-19 diagnosis.
2.Anti-SARS-CoV-2 Spike RBD IgM and IgG serum levels during the 1st week and/or 21 days after symptoms onset by ELISA.
Results: Mild COVID (n=30) showed a cTFH profile similar to HD (n=15). However, we detected a decrease of %cTFH1 (*) but an increase of %cTFH17 (**) in peripheral blood of PIMS infants (n=11) compared to both mild COVID-19 and HD. Indeed, the ratio (cTFH2 and cTFH17)/cTFH1 was increase in PIMS (**). Seropositivity rates were 28% for IgM (n=109) and 25% for IgG (n=337) among children during 1st week of diagnosis, and 63% for IgM (n=51) and 64% for IgG (n=55) after 21 days of symptoms onset. All children with PIMS reached the maximum detectable IgG OD. Interestingly, %cTFH17 positively correlated with anti-SARS-CoV-2 IgG OD (*).
Conclusion: Our results showed that COVID-19 infected children displayed multiple hallmarks of effective humoral response, although the neutralizing activity of antibodies remains to be elucidated. Moreover, the elevated levels of antibodies in PIMS infants point towards their role in severity of disease.

395. (520) **NOVEL FLOW CYTOMETRY IMMUNOASSAY TO STUDY THE PREVALENCE OF ANTI-PROINSULIN AUTOANTIBODIES IN ARGENTINE CHILD-ADOLESCENT PATIENTS WITH TYPE 1 DIABETES MELLITUS**

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2. Servicio de Nutrición, Hospital de Pediatría Ricardo Gutiérrez

Anti-proinsulin autoantibodies (PAA) are often the first markers that appear in patients with type 1 Diabetes Mellitus (T1DM) and its prevalence varies from 10-60% in child-adolescent patients. The gold standard method for PAA detection is the Radioligand Binding Assay (RBA), a highly specific and sensitive technique, but expensive and polluting; thus, it is imperative to develop an alternative method.
The **aim** of this work was studying the prevalence of PAA in Argentine pediatric patients with T1DM using a novel flow cytometric microsphere-based immunoassay (FloCMIA).
Materials and methods: Human proinsulin (PI) was expressed as Thioredoxin fusion protein (TrxPI) in *E. coli* and a fraction was biotinylated. Sera from 100 normal human controls and 51 T1DM patients -all PAA positive by RBA- were used to optimize FloCMIA. A double paratope model was used in which samples were incubated with TrxPI-biotin and microspheres adsorbed with TrxPI. The immune complexes were revealed using streptavidin-Phycoerythrin. The geometric mean of the signals was analyzed, and the results were expressed in Standard Deviation scores. With the optimized FloCMIA, the prevalence of PAA was evaluated in 60 samples of patients with T1DM (age range 0.1-18 years).
Results: The study of ROC curves allowed choosing a cut-off value of 3.7 SDs and the AUC was 0.884, indicating that the method has good ability to distinguish between samples from each group. The specificity of FloCMIA was 97% and the analytical sensitivity 69%, calculated as the percentage of patients RBA positive that were also positive by FloCMIA. There was a substantial agreement between methods (kappa statistic=0.700).
A prevalence of 30% for PAA was obtained in the population of T1DM patients studied.
Conclusions: An alternative method to RBA was developed with good performance and less operational complexity and environmental impact. The novel assay was implemented in Argentine patients with T1DM to study the prevalence of PAA.

396. (529) **PRODUCTION OF RECOMBINANT GAD65 BY INSECT LARVAE AND ITS EVALUATION AS ANTIGEN-DIABETOGENIC SPLENOCYTES PROLIFERATION INDUCTOR**

Marfía JI[1,2], Bombicino SS[1,2], Fuertes F[5], Sabljic AV[1,2], Miranda MV[3,4], Perone MJ[5], Valdez SN[1,2], Trabucchi A[1,2].
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The 65kDa isoform of glutamate decarboxylase (GAD65) is one of the main autoantigens in Autoimmune Diabetes Mellitus.
The aim of this work was to express recombinant GAD65 (rGAD65) in insect larvae and assay its capacity as antigen-driven proliferation of NOD mice-derived splenocytes.
GAD65 was expressed in *Spodoptera frugiperda* larvae using the baculovirus expression system with 97% purity yielding 5.7 mg/g of larvae. rGAD65 immunoreactivity was corroborated by radiometric assay using sera from diabetic patients with antibodies against GAD65. Proliferation assays were performed to evaluate the capability of splenocytes to recognize rGAD65. Splenocytes from pre-diabetic and diabetic NOD mice were cultured in triplicates in 96-well U-bottom plates with RPMI (basal proliferation) or with different concentration of the following diabetogenic antigens: 0.01ug/mL to 1 ug/mL of rGAD65, 0.1 ug/mL to 4 ug/mL of insulin and pancreatic islet lysate and 10 ug/mL of ovoalbumin as negative control. A positive unspecific control was carried out with ConA 10 ug/ml. The cells were cultured for 5 days, [^{3}H]TdR was added in the last 18h of the assay. Cells were harvested and the radioactivity incorporated was determined by liquid scintillation counter. Cell proliferation was expressed as Stimulation Index (SI = antigen-proliferation/basal proliferation). SI obtained for the different doses of each treatment were not significant. Besides, all antigen tested induced proliferation of NOD splenocytes compared to de basal condition ($p<0.01$). SI of pre-diabetic NOD splenocytes ranged from 0.28±0.06 to 2.45±0.25 for rGAD65 at 1 ug/mL to 0.01ug/mL, from 5.34±1.38 to 4.06±0.44 for insulin at 4 ug/mL to 0.1ug/mL and from 5.15±0.03 to 3.58±0.48 for islet lysates at 4 ug/mL to 0.1ug/mL. SI of overt-diabetic NOD splenocytes ranged from 2.06±0.32 to 2.35±0.11, from 3.07±0.19 to 2.95±0.42 and from 2.83±0.28 to 2.01±0.44, respectively. SI for OVA was 0.44±0.6 and 0.57±0.02, and ConA 56.32±5.84 and 15.16±2.52 for pre-diabetic and diabetic, respectively.
In sum, rGAD65 was successfully produced in *S. frugiperda*. Moreover, rGAD65 stimulated diabetogenic splenocytes proliferation obtained from NOD mice, fortunately, similarly to insulin and islets lysate. The dose of 1μg/ml of rGAD65 seems to be toxic for cells. Our preliminary results suggested that rGAD65 can be a potential candidate to generate immunotolerance to prevent experimental autoimmune diabetes.

397. (293) **BONE MARROW TRANSPLANTATION MODIFIES THE RELAPSE TO COCAINE: A POSSIBLE ROLE FOR PERIPHERAL IL-17A SIGNALING**

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Introduction: One of the main challenges to understand drug addic-