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Administration of Amniotic Fluid derived Extracellular Vesicles in COVID-19 Long Hauler Patients

ISEV

particles but also in lighter gradient fractions, suggesting that EVs could contribute to carry the circulating HBV RNA pool. No significant difference was found in NUC-treated vs untreated samples. To further investigate the nature of EVs detected in light density fractions, NTA analysis was performed, showing that these fractions were indeed containing EVs in size spanning from 30 to 150 nm. Finally, proteomic analyses of the same fractions revealed the presence of specific markers of exosomes (CD63, CD9, TSG101 or HSC70).
Summary/Conclusion: Our study will shed light on the molecular biology of serum HBV RNA secretion and will aid the development of serum HBV RNA as a novel biomarker for chronic HBV infection.

OD11.02 | Human cytomegalovirus infection modifies trophoblastic small extracellular vesicles secretion and composition, facilitating viral dissemination in recipient cells

Mathilde Bergamelli, Institut Toulousain des Maladies Infectieuses et Inflammatoires (Infinity), Université de Toulouse, INSERM, CNRS, UPS, Toulouse, France
Hélène Martin, Institut Toulousain des Maladies Infectieuses et Inflammatoires (Infinity), Université de Toulouse, INSERM, CNRS, UPS, Toulouse, France
Jean-Michel Mansuy, CHU Toulouse, Hôpital Purpan, Laboratoire de Virologie, Toulouse, France
Ilse Hurbain, Institut Curie, CNRS UMR144, Structure et Compartiments Membranaires, Université Paris Sciences et Lettres, Paris, France. Institut Curie, CNRS UMR144, Plateforme d'imagerie cellulaire et tissulaire (PICT-IBiSA), Université Paris Sciences et Lettres, Pari
Jacques Izopet, CHU Toulouse, Hôpital Purpan, Laboratoire de Virologie, Toulouse, France.
Graça Raposo, PhD, Institut Curie, CNRS UMR144, Structure et Compartiments Membranaires, Université Paris Sciences et Lettres, Paris, France. Institut Curie, CNRS UMR144, Plateforme d'imagerie cellulaire et tissulaire (PICT-IBiSA), Université Paris Sciences et Lettres, Pari
Daniel Gonzalez-Dunia, Institut Toulousain des Maladies Infectieuses et Inflammatoires (Infinity), Université de Toulouse, INSERM, CNRS, UPS, Toulouse, France
Gisela d'Angelo, Institut Curie, CNRS UMR144, Structure et Compartiments Membranaires, Université Paris Sciences et Lettres, Paris, France
Cécile Malnou, Institut Toulousain des Maladies Infectieuses et Inflammatoires (Infinity), Université de Toulouse, INSERM, CNRS, UPS, Toulouse, France

Introduction: Congenital infection by human Cytomegalovirus (hCMV) is a major public health issue because of its high incidence and the variety of induced neurological sequelae in neonates but despite intense research, pathophysiology of hCMV infection is not yet fully understood. As they participate in mother-fetus communication, we examined the hypothesis that placental small extracellular vesicles (sEVs) could contribute to placental and fetal injury.
Methods: sEV from trophoblastic cells infected or not by hCMV were purified by differential ultracentrifugation and density gradient. sEV structure and composition were further analyzed by flow cytometry, nanoparticle tracking analysis, immune-electron microscopy and proteomics. Finally, impact of trophoblastic sEV on hCMV permissiveness in recipient cells was examined.
Results: We observed that hCMV infection increased secretion of trophoblastic sEV that presented smaller size compared to non-infected condition. Protein content of trophoblastic sEV was modified by hCMV infection, with the presence of viral proteins and with modification in cellular protein composition, suggesting that they could play a role in "priming" infection in recipient cells and thus facilitate further viral infection. Trophoblastic sEV were internalized in fetal cells with time and dose effect. Incubation of fetal cells with sEV from trophoblastic cells infected by hCMV increased significantly the infection rate compared to fetal cell incubated with sEV prepared from non-infected cells.
Summary/Conclusion: In conclusion, we showed that hCMV infection modifies both trophoblastic sEV secretion and protein content, therefore priming fetal recipient cells for a future infection. Hence sEVs may be crucial mediators that could play an important role in maternal-fetal transmission of hCMV by facilitating viral dissemination towards the fetus.

OD11.03 | Administration of Amniotic Fluid derived Extracellular Vesicles in COVID-19 Long Hauler Patients

Maria Ines Mitrani, M.D., Ph.D., Organicell Regenerative Medicine
Michael A. Bellio, Ph.D., Organicell Regenerative Medicine
Gwendolyn Haskell, Pharma D, Organicell Regenerative Medicine
George C. Shapiro, M.D., Organicell Regenerative Medicine

Introduction: Post-COVID-19 infection symptoms such as mental fog, tachycardia, and extreme fatigue are just a few of the symptoms wreaking havoc on patients' lives. Patients with long-term sequelae following COVID-19 are being called long-haulers. To date, long-haulers are receiving little to no guidance from physicians on their lingering COVID-19 symptoms with no treatment options available. Zofin is an acellular biologic that contains the extracellular vesicle (EV) fraction of human amniotic fluid and is under investigation for use as a COVID-19 therapeutic. We have recently completed 4 single patient emergency/compassionate use eINDs investigating amniotic-fluid derived EVs in COVID-19 long haulers under our approved parent IND 19881 to demonstrate safety and feasibility.
Methods: FDA and IRB approval were obtained for these single patient cases investigating Zofin treatment in an outpatient setting. IND approval numbers were: eIND 25888, eIND 26560, eIND 26561, IND 26821. The therapeutic intervention, Zofin, is an allogenic, acellular biologic derived from human amniotic fluid containing 2.3 × 10^11 particles/mL with 70–80% positive expression of exosome markers CD63 and CD81. Zofin was administered intravenous as 1mL doses on baseline, day 4 and day 8 (3 doses). The approved clinical protocol included patient follow up with biomarker testing and chest X Rays (CXR) on Day 0, 4, 8, 14, 21, 28, and 60. The primary objective of these studies was to demonstrate the safety of Zofin. All patients tested positive for COVID-19 a minimum of 2 months prior to treatment.
Results: Administration of the EV product was shown to be safe in all patients. One patient had detectable bilateral pneumonia at baseline treatment that was present 2 months after discharge from the hospital. On Day 14, repeated CXR showed improvement of the patchy peripheral pulmonary opacities. Then, the CXR report on Day 21 noted that the patient's lungs were clear. Furthermore, this patient experienced extreme shortness of breath prior to treatment with baseline pulse oximetry readings were 95% when seated and 93–94% when supine on room air. Improvements in fatigue were noted soon after the second dose and the patient was able to exercise to fatigue, at which time, he did not desaturate and remained at 97–98% on room air.
Summary/Conclusion: The single patient IND studies were completed without any reported adverse events or safety concerns. Furthermore, these completed studies demonstrate the feasibility and a therapeutic potential of amniotic fluid-derived EVs for COVID-19 long hauler intervention.

OD12 | Therapeutics

Chair: Janusz Rak, Professor, Canada
Chair: Shin-ichi Kano, Department of Psychiatry and Behavioral Neurobiology, The University of Alabama at Birmingham School of Medicine, United States

OD12.01 | Tunability of platelet-derived extracellular vesicles

Mari Palviainen, PhD, University of Helsinki
Puutio Johanna, University of Helsinki
Johannes A. Eble, University of Munster
Masood Kamali-Moghaddam, University of Uppsala
Pia Siljander, University of Helsinki

Introduction: The proteome of anuclear platelets comprises >5000 proteins and is impacted e.g. by age and disease. The platelet secretome of over 300 proteins contains e.g. growth factors and immunomodulatory proteins and therefore, platelet products, such as platelet rich plasma are used in regenerative medicine. Platelets also release extracellular vesicles (EVs), both constitutively and upon activation. We studied 1) the tunability of platelet EVs by agonists engaging different critical platelet signaling pathways, 2) the characteristics of these EVs, and 3) macrophage responses to these EVs.
Methods: Isolated human platelets were activated by CRP (engaging GPVI), rhodocytin (CLEC-2), and by thrombin and collagen co-stimulus (all thrombin and collagen receptors). Agonist concentrations and the time of activation were optimized for maximal EV yield in the shortest possible time. EVs were isolated by ultracentrifugation using cushioned density-gradient, and then characterized by particle concentration, size distribution (NTA) and marker protein expression (Exoview R100). The inflammation-linked proteome of EVs was analyzed in a targeted array using Olink technology. Macrophages differentiated from THP-1 cells were treated with equal numbers of EVs for 6 and 24 hours, and the secretome of macrophages was analyzed with Luminex technology targeted for cytokines and chemokines
Results: Although more CD63+/CD9+ EVs were generated from activated platelets when compared to non-activated platelets, activation by rhodocytin resulted in a markedly lower EV yield compared to CRP or TC co-stimulus. The signaling pathways engaged during activation significantly impacted on the inflammation-related molecular cargo. These findings were further supported by the variability of the agonist-, but also time-dependent changes in the secretome of the EV-treated macrophages reflecting the tunability of platelet-derived EVs