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ROLE OF PLATELETS IN LUNG REGENERATION IN MURINE MODEL OF ACUTE LUNG INJURY

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Introduction: This study has been performed within the framework of an international stay (at the Keenan Research Center, St Michael's Hospital, Toronto, Canada) thanks to a mobility aid from CIBERES. The host group works in several research lines in the field of lung regeneration in Acute Respiratory Distress Syndrome (ARDS). After injury, the lung epithelium may activate repair and regeneration for an adequate restoration of epithelial cells or, on the contrary, undergo an aberrant remodeling. Previous studies have shown, in a mouse model of pneumonectomy, that platelets activate lung regeneration (neoalveolarization) through stimulation of alveolar epithelial cells proliferation. Remarkably, lungs have recently been identified as a primary place of platelet biogenesis. Taking this into consideration, we hypothesize that platelets may modulate lung regeneration after ARDS.
Objectives: To study the role of platelets in lung regeneration in mouse models of acute lung injury.
Methods: Two murine models have been used: a model of ARDS by intratracheal HCl instillation (compared to normal saline instillation), which resembles human ARDS by gastric acid aspiration, and a 2-hit model in which, 48 h after acid instillation, high pressure mechanical ventilation was applied for 2 hours, to resemble ventilator induced lung injury. A pilot study allowed us to determine platelet levels in blood and bronchoalveolar lavage fluid (BALF) at different time points, by using a coulter counter and flow cytometry, and to relate it to lung damage. In the acid model we conducted experiments of pre- (previous to the insult) and post- (after the insult) depletion of circulating platelets (compared to non-depleted animals). For estimation of lung damage, albumin and neutrophil infiltrates were assessed in BALF, and lung fibrosis and pathology were/are being scored in tissue by immunostaining; proliferation of alveolar epithelial cells is being estimated on lung tissue images obtained by confocal fluorescence microscopy.
Conclusions: The research conducted has rendered multiple results, which are still under analyses in an ongoing collaboration with the host group. So far, we have successfully optimized and validated the models and the depletion of circulating platelets. Interestingly, BALF platelet counts were not influenced by depletion of circulating platelets. Analysis of the pathology scores and the alveolar epithelial cell proliferation will be crucial to evaluate the influence of circulating platelets in lung repair/regeneration. These results lay the groundwork for a next set of experiments, which could expand the collaboration with the host group.

GUT MICROBIOTA DYSBIOSIS DUE TO HIGH FAT DIET-INDUCED OBESITY MAY BE FAVOURING PROGRESSION TOWARDS ACTIVE TB IN C3HEB/FEJ. THE EXPERIENCE BETWEEN THE EXPERIMENTAL TUBERCULOSIS UNIT AND THE TUBERCULOSIS GENOMIC UNIT

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Introduction: Tuberculosis (TB) is an infectious disease and still a major challenge for the humankind. Several risk factors have been described over the years as diabetes mellitus type 2 (DM2), while others as high body mass index (BMI) have been related to better outcomes in several infectious diseases as TB. In previous studies we have assessed the influence of high fat diet (HFD) in TB development and progression using obese C3HeB/FeJ, and HFD-fed mice showed to have a faster progression towards active TB than those receiving normal diet (ND). In the present study we aimed to analyse the gut microbiota as it has been suggested a link between its composition and the immune system. The existence of this crosstalk could be influencing on the progression towards TB in this subset of animals. In order to evaluate this, the Experimental Tuberculosis Unit (UTE) (CIBERES), located in Badalona, moved to Valencia to the Tuberculosis Genomics Unit (TGU) (CIBERESP) in order to learn how to develop the sequencing process. The group led by Iñaki Comas is focused in the understanding of the biology of the *Mycobacteriumtuberculosis* complex by combining evolutionary, epidemiological, functional and genomic analyses.
Objectives: The aim of this study is to determine the impact of HFD-induced obesity in gut microbiota composition in order to see if it

might have an impact on the development of TB using sequencing methods.
Methods: We used faecal samples collected at weeks 16 and 32 after starting the diet from 2 experiments. In both, we had induced obesity with HFD in C3HeB/FeJ mice strain and applied different conditions to assess their impact on the gut microbiota. In experiment 1, the conditions were: ND vs HFD, single infection (SI) vs multiple consecutive infections (MCI) and no vaccine vs BCG vaccination. In experiment 2, all animals were challenged with MCI and the conditions evaluated were: ND vs HFD, no treatment vs chemotherapy (isoniazid [25 mg/kg] plus rifapentine [10 mg/kg]). DNA from faecal samples was extracted at the UTE. Those samples were sent to the TGU where microbiota composition was analysed by 16S ribosomal RNA sequencing in faecal samples. There, we learned the procedure for the library preparation, the sequencing on Mi-Seq (Illumina) and the further analysis of the result with bioinformatics tools. Our results showed that less number of operational taxonomic units (OTUs) and less diversity have been found in HFD samples. The analysis of most abundant phylum showed reduction in Bacteroidetes and Proteobacteria phylum. Firmicutes phyla did not change but differences in the abundance of some genus inside this phyla have also been found. Chemotherapy has a different effect in the gut microbiota composition of animals receiving ND than those receiving HFD.
Conclusions: Our data showed that HFD causes dysbiosis in the intestinal microbiota and differences in the most abundant phylum, diversity and composition which could influence the faster progression towards active TB we had seen in those animals. Due to this collaboration interciber (CIBERES-CIBERESP), the Experimental Tuberculosis Unit has established a collaboration with TGU and has learned a new technique which has been applied in the study of microbiota composition in many other experiments developed by the UTE after the mobility was done.

PROPERTIES OF SULFIDE-INDUCED CONTRACTION IN RAT PULMONARY ARTERIES

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Introduction: Hydrogen sulfide typically acts as a vasodilator but in some arteries causes constriction or both constricting and dilating phases. Application of H2S elicits a complex contraction in rat pulmonary arteries (PAs) comprising a small transient contraction (phase 1; Ph1) followed by relaxation and then a second, larger, and more sustained contraction (phase 2; Ph2). This response resembles the effect of hypoxia in these arteries. Has been proposed that hypoxic pulmonary vasoconstriction (HPV) is due to a build-up of cellular sulfide concentration during hypoxia resulting from an inhibition of its oxidative metabolism. The mechanisms by which hypoxia constricts PAs remain controversial, but there is substantial evidence that HPV is triggered by increased reactive oxygen species (ROS) production by complex III, leading to sarcoplasmic reticulum Ca2+ release and the opening of store operated Ca2+ channels.
Objectives: Investigate the mechanisms which cause biphasic response using isometric myography in rat second-order PAs, with Na2S as a sulfide donor.
Methods: Male Wistar rats (6-8 week old) were euthanized by lethal injection (intraperitoneal) of sodium thiopental. The heart and lungs were excised and placed in cold physiological salt solution (PSS). Rings of the intrapulmonary artery (inner diameter: 0.5-1.0 mm) were dissected free of adventitia and parenchyma under a dissection microscope, mounted on a conventional small vessel wire myograph, and stretched to give a basal tension of ~5-6 mN (equivalent to an internal pressure of ~15 mmHg). They were then equilibrated with three brief exposures to PSS containing 80 mmol/l KCl. Sulfide was applied to the myograph chamber by adding Na2S in concentrations ranged from 10 to 1.000 M.
Conclusions: The mechanisms of the Ph2 contractile response to sulfide in rat PAs reveal a number of similarities with HPV in the same arteries would be predicted if sulfide also increases ROS production via complex III. However, the observation that rotenone and VAS 2870 exert differential effects on HPV and the sulfide response argues against the concept that sulfide may mediate HPV via this pathway.

LOW-DOSE CHLORINE INHALATION IN HEALTHY HUMANS: A PILOT STUDY

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Introduction: The present study was performed in the Respiratory Department of the Sacré Coeur University Hospital in Montreal, Canada. Chlorine can cause conditions of various severities depending on the concentration inhaled. Chlorine can induce a direct oxidative epithelial injury, but further damage may also occur with a migration and activation of inflammatory cells such as neutrophils within the airway, with a subsequent release of reactive oxygen species and proteolytic enzymes. The release of reactive species can contribute to airflow limitation and airway hyperresponsiveness. The release of matrix metallopeptidases (MMP) and cysteinyl leukotrienes induced by an airway epithelial insult may promote the development of airway remodeling. Scarce studies investigated the effects of low-dose chlorine exposures in humans. The few studies reporting chlorine inhalation tests in humans did not describes, the accuracy and safety of the methods of exposure.
Objectives: The objectives of the present study were: *1)* to describe a closed-circuit apparatus designed to expose humans to chlorine gas in a safe manner; *2)* to investigate the impact of 15 minute-inhalation of 1 ppm of chlorine on airway function and inflammation of healthy humans
Methods: A closed-circuit inhalation challenge apparatus allowing a safe and controlled exposure to chlorine was built. We performed a pilot crossover study including six healthy subjects exposed to gaseous chlorine (1 ppm during 15 min) and fresh air (15 min) with a 2-week washout period between the two periods of exposure. Spirometry, methacholine challenge test and exhaled nitric oxide fraction (FeNO) were measured 24 h before and after exposure. Sputum induction was performed 24h before, 30 minutes and 24 h after exposure. Total and differential cell counts as well as isoprostane, cysteinyl leukotrienes, IFNγ, TNFα, EGF, FGF2, PDGF, VEGF, GMCSF, RANTES, MCP, IP-10, MIP, MDC, FLT-L3, fractalkine sCD40L, IL-1, IL-4, IL-6, IL-7, IL-8, IL-12p70, IL-13IL-15 and, IL-17 were measured in sputum.
Results: Stable levels of 1 ppm of chlorine were reached for 15 minutes in 6 subjects. The subjects were unable to differentiate chlorine exposure from fresh air. No significant change in FEV_1, PC_2O, FeNO levels, sputum cell counts or inflammatory biomarkers was observed after chlorine or fresh air exposure.
Conclusions: We describe for the first time a closed-circuit methodology for a safe, accurate and reproducible exposure to chlorine gas. This system will allow the investigation of the pathophysiology of irritant-induced asthma in humans. Chlorine exposure of 1 ppm for 15 minutes appears safe in healthy individuals as it did not induce any significant change in airway function and inflammation.