Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Revealing lung repair through IL-22 pathway

Synopsis:

Acute Respiratory Distress Syndrome (ARDS) is responsible for 23.4% of intensive care admissions,and is associated with 40% short-term mortality and an increased 5-years morbidity in the survivors. Mechanical ventilation strategies are a main determinant of ARDS outcome, prompting the use of ultra-protective mechanical ventilation in combination with extracorporeal oxygenation (ECMO) for refractory hypoxemic patients. The re-establishment of the alveoli structure involves the differentiation of alveolar epithelial type II cells into type I cells, which are responsible for gas exchange. However, type II cells can also differentiate into mesenchymal cells (epithelial to mesenchymal transition, EMT), which has been linked with abnormal repair process, prolonged inflammation, lung dysfunction, and ultimately death. It is fundamental to better understand lung repair process in order to develop new treatment strategies.

IL-22 targets non-hematopoietic cells, like epithelial and fibroblasts cells, which express high levels of its receptor, a heterodimer consisting of the IL10R2 and IL22R1 chains. Downstream its receptor, IL-22 can signal via STAT1, STAT5, JNK and MAP kinases, although are STAT3 downstream targets that have been linked to epithelial repair in the several epithelium. Through this pathway it induces the production of antimicrobial molecules like regenerating islet-derived proteins (Regs), proliferation signals like SOX2, maintenance of epithelial stem cell niche and inhibition of differentiation toward mesenchymal phenotype.

In experimental models, IL-22 knockout mice exposed to influenza virus have more alveolitis and collagen deposition, and decreased epithelial proliferation. Additionally, IL-22 restores epithelial resistance, suggesting an important role in regulating tight junction expression. Therefore, It is likely its involvement in human epithelial repair upon acute injury.

We hypothesize that IL-22 modifies alveolar type II responses and differentiation to inflammatory and mechanical stimuli.

The main aims of this project will be: 1) To Establish an invitro cell model of alveolar epithelial repair and differentiation of pneumocytes type 1; 2) Assess alveolar epithelial cell response to different mechanical and biologic stimuli modulated by IL-22.

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Work Plan:

We will use A549 cell line and test different differentiation conditions, using TFG-B stimulation or inhibition to differentiate towards mesenchymal cells or type 1 pneumocytes.

Then, we will fill in the scientific gap on the possible modulation by IL-22 of alveolar epithelial cells repair under mechanic stimuli and generated new IL-22 dependent targets to be assessed in vivo. We will create a system capable of mimicking the mechanical power induced by artificial ventilation in cell cultures, simulating cyclic changes in alveolar pressure and shear stress. Taking advantage of the lab experience in cell culture, we will grow human alveolar type II cell lines (A549 cells) in the following conditions: 1) no mechanical stimuli; 2) conventional cross scratching; 3) cyclic changes in pressure; 4) hypoxic and hyperoxia conditions, in the presence and absence of TFGβ1 (an inductor of inflammatory stimuli (IL-1 and IL-6) and IL-22. Experiment read-outs will include flow-cytometer analysis of the expression of pneumocytes type 1 markers (E-cadherin), type II (Prosurfact protein C) and mesenchymal markers (vimentin), as well as STAT3 activation. In parallel, we will analyze cell morphology, and time to fill in the gap in cross scratching experiences. In order to identify genes downstream IL-22 involved in the inhibition of epithelial to mesenchymal transition or differentiation towards type 1 pneumocytes, we will use RNA-seq to compare whole-genome expression profiles, and evaluate the differential expression induced by the several conditions described above, in the presence and absence of IL-22. We select the genes to be tested in vivo based on data mining for: 1) the expected function of a gene, using Gene Ontology of significantly enriched terms associated with Biological Processes and Pathways (GOStats, DAVID, Panther); 2) correlation with evidence from transcriptional profiles of ARDS patient samples, included in ARDS network studies available at NIH repository. Subsequently, we will select molecules that discriminate IL-22 activation, confirm mRNA by RT-PCR. We will validate the expression of these molecules in vivo, in bronchioalveolar samples obtained from patients with ARDS that have been stored in the lab.

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Remunerated or volunteer training: volunteer training (renumeration will be possible)