SPECIFIC ALVEOLAR MACROPHAGE TARGETING IN LPS-INDUCED COPD ANIMAL MODEL USING A FREE-BREATHING NONINVASIVE MR IMAGING PROTOCOL COUPLED WITH THE USE OF ANTIBODY-CONJUGATED SPIO NANOPARTICLES

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Purpose: Coupled with the use of super-paramagnetic iron oxide (SPIO) nanoparticles, MR Imaging of alveolar macrophages (AM) offers a promising noninvasive approach for an early and better assessment of the pathological and physiological impairments in chronic obstructive respiratory diseases (COPD). Inside the body, different environmental conditions will orient macrophages to have either a pro-inflammatory (M1) or immuno-modulator (M2) profile. In this study, the *in vivo* effect of pulmonary administration of SPIO nanoparticles on the polarization profile of AM was assessed and a noninvasive free-breathing MR imaging protocol coupled with the use of biocompatible antibody-conjugated SPIO nanoparticles was developed to allow specific targeting and imaging of a particular subpopulation.

Methods and materials: Balb/c mice were intrapulmonary exposed with bacterial lipopolysaccharides (LPS) to develop a COPD model. They were then exposed with either saline or PEGylated dextran-coated SPIO nanoparticles. Broncho Alveolar Lavage Fluid were collected at 48h post LPS challenge. ELISA, RT-PCR and Flow cytometry analyses were then performed to assess the biocompatibility of the nanoparticles and provide a full characterization of macrophages polarization after LPS and/or SPIO administration. Specific biomarker for M1 and M2 macrophage subsets were selected for selective macrophages targeting. CD86- or CD206-antibodies were conjugated to SPIO nanoparticles to specifically target and image either M1 or M2 macrophages respectively. A free-breathing MR imaging protocol using ultra-short echo time (UTE) radial sequence on a 4.7T magnet was then optimized to allow simultaneous detection of inflammation progress in the lung and detection of macrophages subsets. Flow cytometry and immunohistochemistry analyses were finally performed to confirm MRI readouts and characterize the polarization profile of targeted macrophages.

Results and discussion: While a continuum balance in macrophages subpopulations was identified following LPS challenge, the intrapulmonary administration of the used SPIO nanoparticles, under the current experimental conditions, was found to not affect their polarization profile and to be biocompatible for lung administration in pre-clinical settings. The use of UTE radial sequence allowed obtaining enough MR signal in lung parenchyma and sensitively detecting the presence of inflammatory regions homogenously distributed in the LPS induced COPD lung. M1 and M2 macrophages were successfully targeted using CD86- and CD206-conjugated MNP respectively and detected in the lung as hypo-intensity void signal dots using a free-breathing imaging protocol (Figure1). Macrophages were also found to co-localize with inflammatory regions induced by LPS challenge (Figure2). No variation in the polarization profile of targeted macrophages were observed even though a continuum switch in their polarization might occurs.

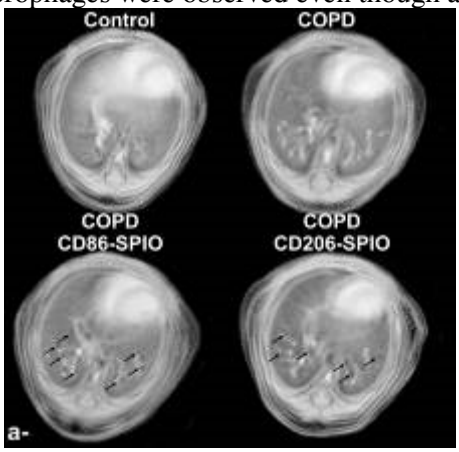

Figure1: *MR Images of a control, COPD (48h post LPS-induction), COPD + CD86-SPIO and COPD + CD206-SPIO groups (2h post-intrapulmonary administration of either CD86- or CD206-conjugated SPIO nanoparticles).*

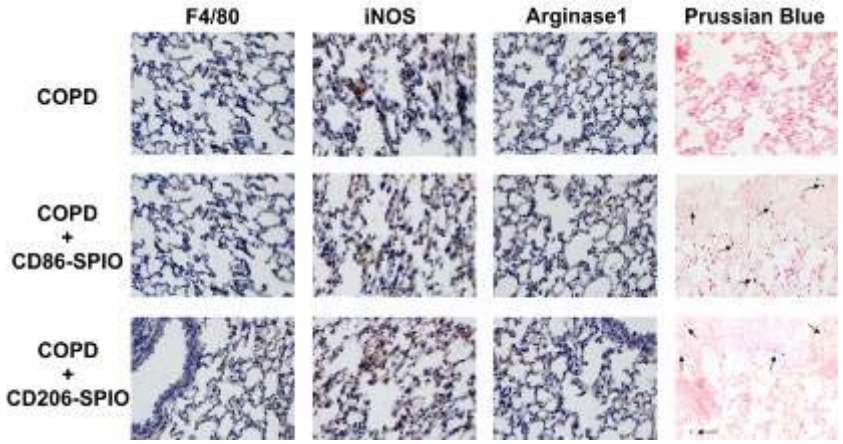

Figure2: *Immunohistochemistry (IHC) analysis of F4/80 (universal marker of macrophages), iNOS (marker for M1 macrophages), Arginase1 (marker for M2 macrophages) and Prussian Blue (marker for iron oxide) staining in COPD lung before and after intrapulmonary administration of either CD86- or CD206-SPIO nanoparticles.*

Conclusion: AM polarization was extensively characterized after LPS and/or SPIO administration. Specific biomarkers targeted to either M1 or M2 macrophages subpopulations were identified. Biocompatible antibody-conjugated SPIO nanoparticles were then reported as a novel approach to specifically target one subpopulation of macrophages in the lung of a COPD animal model and allow their noninvasive tracking using a free-breathing MRI protocol. Flow cytometry and immunohistochemical analysis confirmed MRI readouts and suggested a balance in M1 and M2 macrophages after pulmonary administration of the nanoprobes that can serve as a novel diagnostic and therapeutic agent.