Imaging an inflammatory response in a sponge granuloma model using ^{19}F-MRI

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Introduction: Accumulation of monocytes/macrophages is the hallmark of chronic inflammation. The ability to monitor this response non-invasively, in vivo, may facilitate development of new therapies. V-Sense® (Celsense, Inc., Pittsburgh, PA), a ^{19}F-based MRI tracer agent, is a perfluorocarbon nanoemulsion that is internalized by macrophages following intravenous injection[1,2]. Perfluorocarbons have higher specificity than traditional MRI contrast agents, as there is no endogenous fluorine in the body, and the MR signal is a direct measure of the agent concentration[3]. We used ^{19}F MRI to detect an inflammatory response in a sponge granuloma model and identified the types of cells involved in this response using Fluorescence Activated Cell Sorting (FACS).

Materials & Methods: *Inflammation model:* An inflammatory response was induced in mice using PVA sponges that were soaked in either complete Freund's adjuvant (CFA) or PBS (control) and implanted subcutaneously in the dorsal surface of BALB/c mice (day 0). V-Sense (0.2 ml) was injected intravenously on day 4. *Imaging:* Mice were imaged on days 5 and 6 at 7 Tesla using a 72 mm diameter, dual ^{19}F/^{1}H quadrature birdcage coil (m2m Imaging Corp., Cleveland, OH). A ^{1}H anatomical scan was acquired using a spin-echo sequence with slices covering the sponge, liver and kidneys. A ^{19}F scan was then acquired over the same slices using a fast spin echo sequence: TR/TE=4000/12.5 ms, 8 echoes per excitation, k space centered on the 4th echo, 120 averages, 64x64 matrix, 28x28mm FOV, and 1.5mm slice thickness. *FACS analysis:* V-sense was labeled with DiI, a fluorescent dye. Cells collected from the sponges were stained with saturating amounts of antibodies specific for Ly6G (FITC-conjugated) and CD11b (PE-Cy5-congugated) as labels for macrophages and neutrophils respectively. Stained cells were analyzed on a flow cytometer and fluorescence and forward light scatter signals were collected on 100,000 cells. The percentage of cells with a particular fluorescence pattern was determined by integration of the curves for corresponding populations.

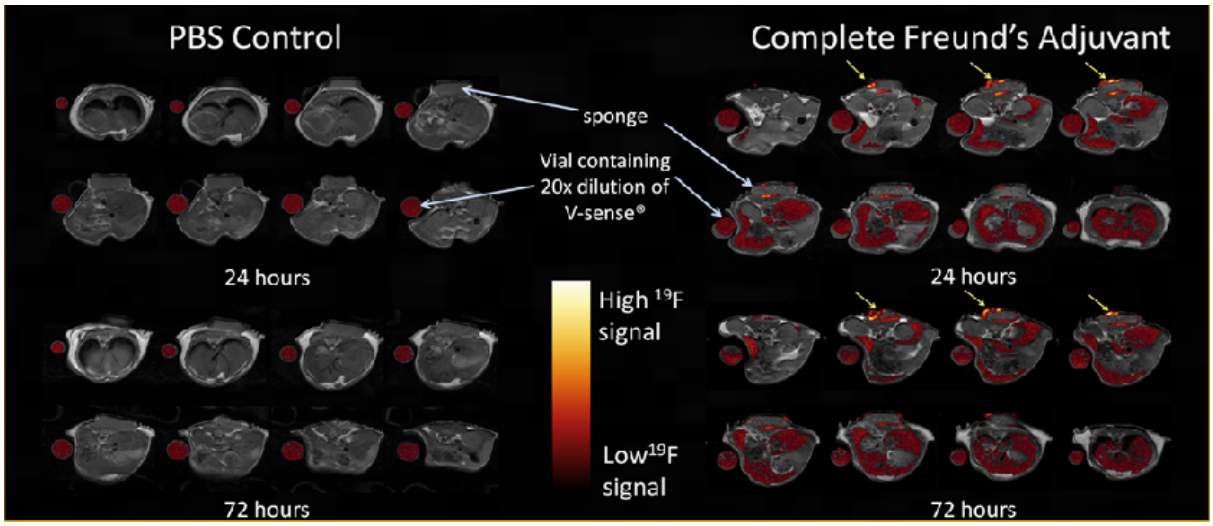

Figure 1: Fluorine-19 MRI signal, indicated by the HOT color overlay on grayscale anatomical MRIs, was not detected in animals implanted with PBS soaked sponges (Left). Animals implanted with CFA-soaked sponges (Right) showed high ^{19}F signal in sponges (arrows) and in the liver. Animals implanted with CFA soaked sponges accumulated over 10x the number of inflammatory cells relative to control animals.

Results & Discussion: Significant ^{19}F signal was observed surrounding the CFA-soaked sponges, consistent with macrophage accumulations (**Fig. 1**). In contrast, no ^{19}F signals were detectable in the control PBS-soaked sponge animals. FACS analysis showed that macrophages predominate the PBS sponges, while neutrophils predominate the CFA sponges. Further, two populations of cells uptake the ^{19}F nanoparticles in CFA sponges: (i) A CD11b+/Ly-6G- population, identified as macrophages. (ii) A CD11b+/Ly-6G+ population of very large cells that may be dendritic cells. The positive ^{19}F signal in the livers of the CFA treated animals suggest that V-sense may be specific to an activated macrophage subtype.

Conclusion: These preliminary results indicate that the V-Sense ^{19}F-based nanoemulsion is a specific in vivo biomarker for inflammation that can potentially be used to quantify macrophage activity. Local inflammation with CFA results in a systemic change in macrophage phenotype as evidenced by the accumulation of ^{19}F nanoparticles in the liver of CFA sponge bearing animals, but not in those implanted with PBS sponges.

References: **1.** Srinivas et al. Magn Reson Med. 2007 Oct;58(4):725-34. **2.** Hitchens et al., Abstract #1688, ISMRM 2008. **3.** Yu JX et al., Curr Med Chem. 2005;12:819–848.