Quantitative assessment of macrophage activity in inflammatory bowel disease using fluorine-19 MRI and the response to treatment

D. K. Kadayakkara1,2, and E. T. Ahrens1,2
1Carnegie Mellon University, Pittsburgh, PA, United States, 2Pittsburgh NMR Center for Biomedical Research, Pittsburgh, PA, United States

Introduction
Macrophages play an important role in the induction and progression of inflammatory bowel disease (IBD) (1). Longitudinal, non-invasive imaging of macrophage activity can be an important diagnostic tool in assessing disease progression and response to treatments. In this work, we characterize inflammatory burden in a mouse model of IBD using in situ macrophage labeling with perfluorocarbon emulsion (PFC) followed by 19F MRI detection. 19F signal was highly specific to macrophages in the colon, with no background signal, and linearly proportional to inflammation severity. We demonstrate that the 19F signal, and hence macrophage activity in the gut, decreased in mice treated prophylactically with cyclosporine A (CsA), but increased in mice treated with dexamethasone. Results were validated using ex vivo MR microscopy, confocal microscopy, histology, and qRT PCR.

Methods
IBD was induced in IL10-/- mice (n=16) by feeding piroxicam-doped chow for 14 days. Five of these mice received dexamethasone (1.2 mg/kg i.p daily for 14 days) and five of them received CsA (10 mg/kg i.p daily for 14 days). PFC emulsion (VS-1000H, Celsense, Pittsburgh, PA) containing a trace amount of DiI fluorophore in the surfactant was injected i.v. (0.2 mL) via the tail vein. Anesthetized mice were imaged two days after PFC injection. Multi-slice, 1H MRI data were acquired at 11.7 T through the abdomen, along with co-registered 19F images. 19F signal intensity in the colon was quantified relative to an external PFC reference tube using Voxel Tracker software (Celsense). Excised colon tissues were also imaged ex vivo using MR microscopy. Colon tissues were subjected to H&E histology, immunohistochemistry to look at macrophages (F4/80), neutrophils, monocytes (Ly6C) and endothelial cells (CD31), and RNA was extracted to measure macrophage load using qRT PCR.

Results and conclusions
Thickening of the colon wall was observed in 1H images, and patchy 19F signals were observed (Fig. 1a). H&E staining displayed pancolitis with heavy mononuclear cell infiltration. Immunofluorescence of colon tissues showed that PFC was localized exclusively within macrophage (Fig. 1b). The qRT PCR revealed a linear correlation between macrophage RNA and 19F signal in the same tissue samples (R=0.65, p=0.03) (Fig. 1c). The colon 19F signal was reduced in CsA treated animals and increased in dexamethasone treated animals in all regions of the colon (Fig. 1d), reflecting changes in macrophage burden. The 19F data was validated by histology scores in the colon. Our results demonstrate that 19F MRI is an effective method to quantitatively measure macrophage activity in vivo and assess treatment response in IBD.

Fig. 1. MRI-based analysis of macrophages in IBD model. (a) 1H/19F axial composite image through the abdomen showing PFC accumulation (red) in ascending and descending colon walls (arrows). R represents the reference tube containing PFC. (b) Immunohistochemistry of colon tissue where macrophages engulfing PFC-DiI (F4/80 macrophage is green, PFC-DiI is red, nuclei are blue). (c) qRT PCR results showing linear correlation plot between 19F signal and macrophage mRNA in the same colon segments (R=0.65, p=0.03). Expression of macrophage mRNA CD68 is normalized with expression of mRNA of a house keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (d) Quantitative analysis shows an increase in 19F signal in dexamethasone treated animals (n=5) and a decrease in cyclosporine treated animals (n=4) in ascending colon (asc.), descending colon (desc.) and ileocecal region of the colon compared to untreated controls (n=5).

References

Acknowledgements
We thank Lisa Pusateri for technical assistance and Dr. Won-Bin Young for helpful discussion. This work is funded by NIH R01-CA134633, R01-E003453, P41-E001977.