

Assessment of Renal Inflammatory Cell Infiltration in a Murine ANCA-Induced Glomerulonephritis Model by ^{19}F -MRI

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Target audience: Preclinical MR researchers, imaging scientists, nephrologists, immunologists and clinical scientists with an interest in assessing renal inflammation.

Introduction and Purpose: Anti-neutrophil cytoplasmic antibodies (ANCA) are responsible for the development of small vessel vasculitis. A primary concern in clinical practice is the renal involvement, since activation of neutrophils and monocytes by ANCA causes glomerulonephritis (necrotizing and crescent-shaped proliferative glomerulonephritis, NCGN). Currently, diagnosis of the initial inflammation and its recurrences relies on kidney biopsies, which are associated with potential complications and cannot be carried out repetitively. Hence, the clinical need for non-invasive methods which support serial and longitudinal investigations. MRI of fluorine-labeled immune cells has previously been applied to assess inflammatory activity in rodent models of kidney allograft rejection.¹ We have recently generated and employed multi-modal fluorescently (DiI) and ^{19}F labeled nanoparticles to identify specific inflammatory cell populations in an animal model of brain inflammation.² By applying this approach in an established ANCA glomerulonephritis animal model we tested the hypothesis that ^{19}F MRI after in-vivo ^{19}F -labeling of monocytes and neutrophils (i.v. injected perfluorocarbon, PFC) allows non-invasive detection of ANCA-induced renal inflammation.

Methods: Nanoparticle phagocytosis analysis: Feasibility of in-vivo ^{19}F -labeling and tracking of neutrophils and monocytes was tested in a murine model of peritoneal inflammation after i.p. injection of thioglycolate. ^{19}F nanoparticles were injected i.v. (100 ml of 50 mM, particle size 153nm), peritoneal neutrophils/monocytes were purified 4h/48h after peritonitis induction and the ^{19}F signal then quantified ex-vivo by MR spectroscopy. For in vitro experiments double-labeled (^{19}F , DiI) particles were used. **Animal model:** An established mouse model of ANCA-induced glomerulonephritis was used, in which MPO-deficient mice (n=5) were immunized with murine MPO, followed by irradiation and bone marrow transplantation (BMX) of MPO-positive wild-type bone marrow. These animals developed severe ANCA-induced glomerulonephritis, starting 4 weeks after BMX. Control groups consisted of mice that only underwent BMX (n=5) and naïve mice (n=2). **MR imaging:** ^{19}F nanoparticles were injected i.v. 24 hours and 4 hours before MRI. In-vivo ^{19}F spin-echo images (3D RARE, TR = 800ms, TE = 6ms, spatial resolution = $0.94 \times 0.94 \times 1.88\text{mm}$, NEX = 256, TA = 55min) were acquired on a 9.4T Bruker Biospec (Ettlingen, Germany) using a $^1\text{H}/^{19}\text{F}$ volume resonator (inner diameter 35mm). For high resolution ex-vivo MRI the protocol was adapted to a spatial resolution of $(94 \times 94 \times 188)\mu\text{m}^3$. MRI was conducted in-vivo under isoflurane anesthesia, in naïve animals or 8 weeks after BMX. All in-vivo ^{19}F MR images were automatically scaled to the same intensity range (SNR 4 to 30).

Results: Eight weeks after BMX the animals developed a severe glomerulonephritis ($17.4 \pm 3.5\%$ crescents, 9.9 ± 1.8 necroses, n=9). Immunocytochemistry showed a monocytic and neutrophilic infiltration (0.35 ± 0.23 monocytes/glomerulus vs. 0.10 ± 0.05 neutrophils/glomerulus). In-vivo ^{19}F MR images of the mouse abdomen (Fig. 1) showed extensive inflammatory cell infiltration into the kidney as phagocytized ^{19}F nanoparticles in ANCA-induced NCGN BMX mice (Fig.1 left), less infiltration in control BMX mice (Fig.1 center) and no infiltration in healthy naïve mice (Fig.1 right). Signal artifacts outside of liver, spleen and kidney were partially due to motion artifacts since no respiration trigger was applied for reasons of acquisition time shortening. Ex-vivo high resolution $^{19}\text{F}/^1\text{H}$ MRI revealed a strong fluorine signal within the renal cortex and outer medulla (Fig.2). Assessment of the engulfment of nanoparticles phagocytes revealed a more efficient labeling of monocytes as compared to neutrophils by MR spectroscopy (neutrophils 2.74 ± 0.25 vs. monocytes 25.79 ± 3.18 a.u.) and by fluorescence-activated cell sorting (FACS, Fig.3).

Discussion and Conclusion: Renal inflammatory cell infiltration in a murine ANCA-induced glomerulonephritis model was successfully detected by ^{19}F -MRI. The use of this method requires the phagocytosis of the ^{19}F nanoparticles by myeloid cells. ^{19}F nanoparticles conjugated with DiI-fluorescence allowed quantification of phagocytosis by myeloid cells using flow cytometry. Flow cytometry revealed that particle phagocytosis by monocytes was significantly higher versus neutrophils. Although these observations suggest that the ^{19}F signal originates predominantly from labeled monocytes, ultimate statements about the origin of the ^{19}F signal require further corroboration. Notwithstanding this caveat, our data suggest that ^{19}F -MRI may represent a novel non-invasive method for the assessment of renal inflammation in mouse models of ANCA-induced glomerulonephritis with the potential of translation into a clinical tool for the diagnosis of glomerulonephritis in patients.

References: [1] Hitchens T, et al. (2011) MRM 65(4):1144. [2] Waiczies H, et al. (2013) Sci.Rep. 3:1280.

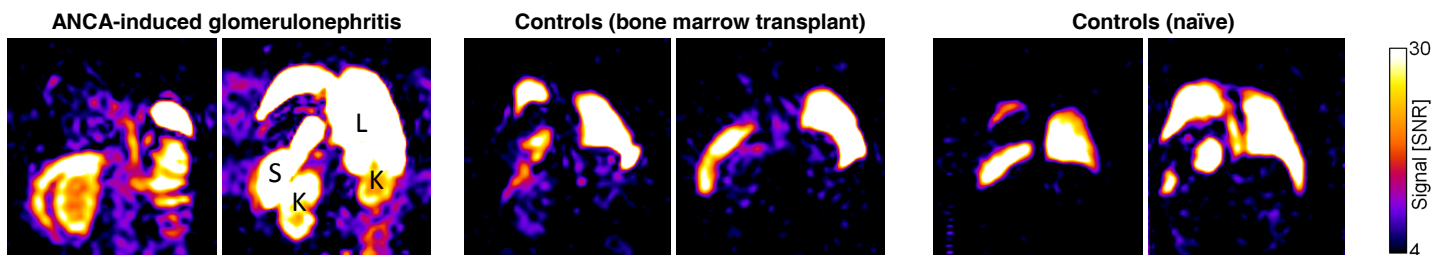


Figure 1: Coronal ^{19}F images of 6 mice. **Left:** mice with ANCA-induced glomerulonephritis (NCGN, 8 weeks after BMX) showed extensive renal inflammatory cell infiltration, particularly in the cortex. (L)liver, (S)spleen, (K)kidneys. **Center:** control mice that underwent BMX alone merely showed a very weak signal in renal cortex 8 weeks after BMX (besides the usual signal in liver and spleen). **Right:** naïve mice showed a high signal in liver and spleen, but none in the kidneys.

ANCA-induced glomerulonephritis (ex-vivo)

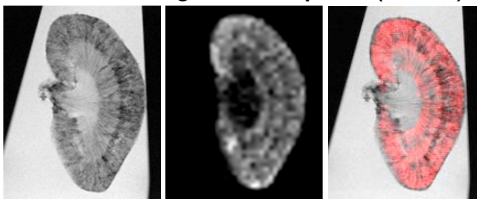


Figure 2: Ex-vivo MRI of kidney from a mouse with ANCA-induced glomerulonephritis. ^1H image (left, $63\mu\text{m}$ resolution), ^{19}F image (center, $250\mu\text{m}$ resolution) and $^{19}\text{F}/^1\text{H}$ color overlay (right).

Phagocytosis of DiI-labeled ^{19}F particles

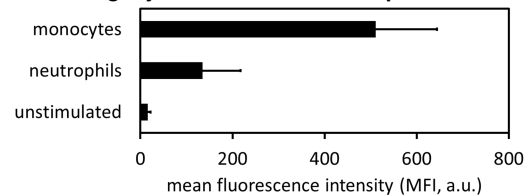


Figure 3: FACS demonstrated more efficient phagocytosis of DiI-labeled ^{19}F particles by murine monocytes vs murine neutrophils in whole blood.