A matrigel-based lipopolysaccharide release model to evaluate the versatility of different MRI approaches for detection of inflammation

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Introduction: The visualization of inflammatory processes still poses a serious challenge, because especially in the initial phase the affected tissue does not exhibit specific physical properties that can be used to create contrast between inflamed and healthy regions. Several MR techniques like T2-weighted MRI, late gadolinum enhancement, SPIOs or ^{19}F MRI have been applied for detection of inflammation [1, 2, 3]. However, but it is still unclear which of these techniques provide the most reliable sensitivity and specificity. Furthermore, current models for inflammatory processes associated with glomerulonephritis, inflammatory bowel disease, transplant rejection, neurodegenerative brain diseases, or myocarditis are very complex and are characterized by a large variability which further complicates the evaluation of the different techniques. We therefore developed and characterized a matrigel-based lipopolysaccharide (LPS) release model for standardized inflammation. Matrigel is an extracellular matrix like substance which is a fluid at room temperature and converts into a gel at body temperature, resulting in a defined structure after subcutaneous injection. Lipopolysaccharide (LPS) is a component of the outer membrane of gram negative bacteria and stimulates innate immune responses via the TLR4 pathway. In a first step, we aimed to detect inflammation in this model by T2-weighted 1H MRI as well as by ^{19}F MRI after application of perfluorocarbons (PFCs) as contrast agent. Furthermore, we validated this model by analysis of immune cell filtration by histology and flow cytometry.

Methods: Matrigel/LPS (50 µl matrigel with LPS: 0, 1, 10 or 50 µg) was injected subcutaneously into the neck of 8-10 week old male C57BL/6 mice. MRI was performed at a Bruker DRX 9.4T NMR spectrometer equipped with a Bruker microimaging unit (Micro 2.5) using a 25-mm ^{1}H/^{19}F birdcage resonator. Mapping of T2 relaxation times was done by a multislice multiecho sequence (FOV 2.56x2.56 cm, matrix: 128x128, slice thickness 0.5 mm, TE = 40 ms, 8 echos, scan time 8 min) and analyzed by an in-house developed software tool. Anatomical ^{1}H reference images and ^{19}F images were acquired using RARE sequences (^{1}H: FOV 2.56x2.56 cm, matrix size 128x128 or 256x256, slice thickness 0.5-2 mm, scan time 1-4 min; ^{19}F: matrix size 64x64, FOV 2.56x2.56 cm, slice thickness 1-2 mm, scan time 21 min). For ^{19}F labelling of monocytes/macrophages, PFC emulsion (500 µl of 10 % perfluoro-15-crown ether w/v) was injected intravenously via the tail vein 24 hrs after matrigel implantation. ^{19}F MRI was performed 4d after PFC application, to ensure optimal deposition of ^{19}F-labeled immune cells in the infected area [4]. To analyze immune cell infiltration by flow cytometry, matrigel plug was excised and digested in a collagenase/DNAse solution at 37 °C for 30 min. Cells were stained with mAbs against CD45, CD3, B220, CD11b, Gr1, Ly6c, CD11c, MHCII and F4/80 to detect T-cells, B-cells, neutrophil granulocytes, macrophages/DCs. For immunohistology plugs were embedded in Tissue-Tek, 8 mm sections were cut and stained with an anti CD11b mAb. Nuclei were counterstained with DAPI.

Results and Discussion: Subcutaneous implantation of matrigel resulted in a defined ellipsoid structure which could be visualized by ^{1}H MRI and enabled exact anatomical location (Fig. 1A). Addition of 50 µg of LPS to the matrigel resulted in a macroscopically visible local inflammation, whereas control plugs did not show any signs of inflammation. Interestingly, determination of the T2 relaxation times in the LPS-containing and control plugs showed a reduction in T2 in the inflammatory plug compared to controls (98.9+/-4.4 ms vs. 69.4+/-8.2 ms, n=3 each). Injection of PFCs for loading of immune cells with ^{19}F label enabled us to track specifically the infiltration of these cells into the affected area. ^{19}F MRI of control matrigel plugs 4d after PFC injection did not result in any measurable ^{19}F signal. In contrast, ^{19}F signal in the LPS containing plugs could be readily detected, was proportional to the amount of LPS applied, and was restricted to areas in the periphery of the matrigel plug (Fig. 1B). Subsequent immunohistochemistry confirmed the predominant localization of CD11b+ immune cells in the boundary area of the plug next to normal tissue. Furthermore, flow cytometry revealed only a small amount of immune cells in control plugs but a pronounced infiltration of monocytes and granulocytes (CD11b+) into LPS containing plugs. *Ex vivo* incubation of murine blood with FITC labeled PFC emulsion particles showed a strong uptake by monocytes, neutrophil granulocytes and B-cells but not T-cells, indicating that the immigration of PFC-labeled monocytes and granulocytes were responsible for the observed ^{19}F signal. Flow cytometric analyses of immune cells 5d and 18d after implantation showed a decrease in the total amount of leukocytes (Fig. 1C). Moreover, the relative amount of neutrophil granulocytes decreased whereas the amount of macrophages/DCs and T-cell number increased from 5d to 18d, refecting the transition from the acute to the resolution phase of inflammation.

Conclusion

Matrigel releasing LPS is a standardized and quantitative model for induction of subcutaneous inflammation which mimics the full course of inflammation from the acute to the healing phase. Subcutaneously implanted matrigel/LPS is detectable by ^{1}H- and ^{19}F MRI which enables exact anatomical location of the inflammation hot-spot. Surprisingly, T2 relaxation times were significantly decreased within the inflammatory region. Concomitant acquisition of ^{19}F images after PFC loading of immune cells enabled a specific and quantitative detection of inflammation as confirmed by flow cytometric and histological analyses. Our matrigel/LPS model is a suitable method to assess and optimize the performance of different MR techniques for noninvasive visualization of inflammation.

References:

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Figure 1: (A) ^{1}H MRI of the neck area after injection of 50 µl of PBS (control) or 50 µl of matrigel. Arrows indicate the site of injection. (B) ^{19}F signal is only detected in LPS containing matrigel plugs (50 µg LPS) and not under control conditions 5d after implantation (C) Analysis of immune cell infiltration after 5d and 18d in control (5d only) and LPS containing matrigel plugs.