Quantification of Dendritic Cells entering the Lymph Nodes in Mice using 19-F MRI

Conrad Steven Martin1, Min-Chi Ku1, Stefano Lepore1, Helmar Waiczies2, Andreas Pohlmann1, Jan Hentschel1, Matthias Dieringer1,2, Thoralf Niendorf1,2, and Sonia Waiczies1,3

1Berlin Ultraschall Field Facility (B.U.F.F.), Max Delbrück Center for Molecular Medicine, Berlin-Buch, Berlin, Germany, 2MRI.TOOLS GmbH, Berlin, Germany, 3Experimental and Clinical Research Center, Berlin, Germany

Introduction:
Fluorine-19 (19F) can be used for the labeling and tracking of cells in living organisms using MRI. Perfluorocompounds (PFC) are very stable; however, they are lipophilic and need to be emulsified to be taken up by phagocytes. Following labeling, 19F-labeled cells can be applied in vivo to follow their movement over a period of several days or even weeks. With the advent of high field MRI, and with the corresponding increase in signal sensitivity it is possible to track even small numbers of cells in very specific locations in vivo [1]. In this work the goal was to quantify numerically and temporally the movement of mouse dendritic cells (DCs) to the popliteal lymph nodes after intradermal injection. This is relevant to immunological research since it gives a longitudinal understanding of DC migration in vivo. DC migration has commonly been studied in cross-sectional studies. By studying the longitudinal behavior of DCs in vivo, as well as ways and means how this behavior can be manipulated, attempts are created to deliver effective human DC vaccines in tumor and infectious disease.

Method:
DCs were generated from bone marrow of C57BL/6 mice as previously described [2]. Nanoparticles containing Perfluoro-15-crown-5-ether (PFCE) were produced in a size range of 300-350 nm. These nanoparticles were applied to DC cultures on day 9 (10 µmol of 19F per culture plate equivalent to approximately 107 cells), which resulted in a quantity on the order of 3 to 10 nmols of 19F per million cells. These cells were matured overnight using lipopolysaccharide and harvested on day 10 of the cell culture, then injected intradermally into the footpads of mice (5 to 10 million cells per footpad). The mice were subsequently scanned in a 9.4 Tesla small bore scanner (Bruker Biospin, Ettlingen, Germany) equipped with a 35 mm 19F RF coil. Phase cycled balanced steady state free precession (bSSFP, 1 average, TE=1.772 ms, TR=3.595 ms, scan time=1:02, 4 phase cycles) scans were performed to achieve high contrast images of the lower limb, importantly to distinguish the popliteal lymph nodes from the surrounding tissue. Point resolved 19F spectroscopy (PRESS, TE = 11.64 ms, TR = 1500 ms, voxel size (5x5x5) mm3, NEX= 512, scan time = 13:54min). Voxels were placed on the popliteal lymph node after voxel based B1 shimming was performed. 19F-PRESS measurements were conducted between 3 and 8 hours after injection. For calibration of the in vivo scans 19F spectroscopy was conducted with a known number of cells from the cell culture in order to determine the exact concentration of fluorine in the cells. A plot is constructed of the numbers of cells in each 19F-PRESS voxel scan vs. the amount of time after injection. To assure correct flip angles for 19F-PRESS, normalized radio frequency transmission field (B1) maps were acquired using the double angle method (α=45°, α=90°, slice thickness=7 mm, TE=2.8 ms, TR=7000 ms, scan time=7:30 min).

Results:
Coronal and axial B1+ maps show rather uniform 19F transmission field uniformity as demonstrated Figure 1A and 1B. Mean B1+ deviation between left and right legs was ±0.1 and ±1.4 respectively (normalized values), which allows a comparison. An overlay of both 19F (red) and 1H (gray scale) bSSFP images indicates the spatial distribution of 19F labeled DC and the anatomy of the lower limb, respectively (Figure 2A). Typical spectral peaks of the 19F signal from PRESS voxel scans of lymph nodes are shown in Figure 2B. The threshold of detection in the PRESS voxel scans lies at approximately 10,000 cells (Figure 2B), depending on the unique concentration of PFCE taken up by the cells from that specific culture. Due to biological reasons, only a small percentage of the applied DCs actually reach the popliteal lymph nodes 5-6 hours after injection (standard deviation of 1.5 hours) as illustrated in Fig. 2C. The cell movement to the popliteal lymph node usually reaches a maximum at 250,000 cells (standard deviation of 150,000 cells) approximately 7-9 hours after injection. After this point the cell concentration in the lymph node decreases until after 72 hours no more 19F signal is detectable on the bSSFP scans. At this point the cells most likely have either moved beyond the popliteal lymph nodes to other lymph node stations, or the cell debris has metabolized via the reticuloendothelial system.

Discussion and Conclusions:
Our results demonstrate that it is feasible to track and quantify the movement of 19F-labeled DCs in vivo in mice over a period of several hours and even days. Although the standard deviations of the number of cells observed and of the timeframe in which they were observed is quite large, their consistency is sufficient to make distinctive claims about the cellular movement of DCs in vivo. Given a sufficiently large sample size, experiments can be done using this procedure to test cell types with various genetic and biochemical modifications for their mobility and direction in vivo. This will be extremely useful in immunological studies involving the genetic and chemical modifications of human cells for the treatment of various tumor diseases such as Glioma, which cannot easily be reached by surgical or chemotherapeutic means. Although there is always a significant degree of variation in the biological functions of different animals in vivo, it is possible to improve the consistency of the cellular motion observation with more sensitive scanning equipment, and by gaining more precise and consistent control of culturing techniques, nanoparticle production techniques, and injection procedures. Such a reduction in procedural variables would benefit the accuracy to determine the effect of various cellular modifications on their resulting mobility and accumulation points inside of test subjects.

References: