Non-invasive monitoring of human dendritic cell migration in the CB17 scid mouse by cellular MRI

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Introduction: The successful migration of adequate numbers of *in vitro*-generated human dendritic cells (DC) from the site of injection to a draining lymph node is a necessary and crucial step in order for a DC-based vaccine to be a successful immunotherapy for cancer and infectious diseases. Currently, less than 5% of injected DC migrate to a draining lymph node. Since the quality and magnitude of an immune response is directly related to the number of antigen presenting DC in the lymph node it is important to develop methods to improved DC migration to a target lymph node. In order to improved the efficacy of DC-based immunotherapeutic vaccines it is necessary to track and assess DC migration to a lymph node. Thus, a reliable and non-invasive method is required to monitor human DC migration in preclinical animal models and subsequently in humans. In this manner it will be possible to optimize experimentally the culture conditions and the manner in which DC are delivered at the site of injection that lead to optimal numbers of DC arriving in a patient's target lymph node. How well a preparation of DC migrates is best assessed by conducting migration assays *in vivo*. A pre-clinical model to assess human DC migration would very useful in this regard. Here we demonstrate that *in vitro*-generated human DC can be readily labeled with superparamagnetic iron oxide (SPIO) nanoparticles and, following injection, their migration to the popliteal lymph nodes (LN) of immuno-compromised CB17 *scid* mice can be monitored. This preclinical model to evaluate human DC migration should provide a better and more reliable means to evaluate and optimize DC-based vaccines prior to conducting a clinical trial.

Methods: ***Human dendritic cell generation, labeling, and footpad injection***: Negatively selected, human monocytes were cultured with GM-CSF and IL-4 and then matured on day 6 with TNF-α. On day 7 cells were labeled with SPIO overnight. On day 8, DC were labeled with membrane dyes PKH26 or CFSE and injected into hind footpads of adult male CB17 scid mice. Injected into the right footpad were $1x10^6$ SPIO and PKH26/CFSE labeled DC while only PKH26/CFSE-labeled DC were injected into left side. DC were derived from 3 normal human and 3 cancer patient samples.

Cellular MR imaging: Forty-eight hr after DC injection, cellular MR imaging was conducted with a 1.5 T GE CV/i MR scanner, using a custom-built gradient coil (inner diameter = 12 cm, maximum gradient strength = 600 mT/m, and peak slew rate of 2000 T/m/s) and a customized solenoidal radiofrequency coil (diameter = 4 cm, length = 4.5 cm). The scanning acquisition (FIESTA) was applied at a resolution of $200um^3$ isotropic over a 5 cm FOV using radio frequency phase cycling. Zero filling was used to obtain an interpolated voxel dimension of $100um^3$. Acquisition time was < 25 min.

Identification of human DCs in the popliteal lymph node: After the MRI scanning, the popliteal LN were removed, fixed, cryoprotected, embedded in OCT compound, and cut into 12 um sections. The presence of human DC in the popliteal LN were identified by: (1)PKH26 or CFSE red fluorescence; (2) Perl's Prussian blue staining; (3) reactivity to human CD45 by immuno-histochemical staining (green fluorescence) with nuclei were stained with DAPI (blue fluorescence) and by flow cytometric analysis of cells positive for human CD45, human CD11c and CFSE.

Results: Normal human (Fig. 1A, top row) and cancer patient SPIO-DC migrated to the popliteal lymph node of CB17 mice as identified (at 48 hr post-injection) by the presence of signal voids in the right LN but not left control LN following a cellular MRI scan. In right LN the distribution of PKH26 (Fig1A, middle row) and Perls Prussian blue for iron (Fig 1A, bottom row) overlapped. LN also contained cells that were $PKH26^+huCD45^+$ double positive (Fig. 1B). This was confirmed by flow cytometric analysis of LN (Fig. 1C). The majority of the PKH26+huCD45 staining and Perls staining for iron was located along the peripheral edges of the LN although some more centrally located cells were also present. The latter was enhanced if the mice were first irradiated and then injected with human lymphocytes and granulocytes left over from the negative selection of the monocytes the day they were isolated and allowed to repopulate the CB17 mice for 8 days while the DC were being derived in culture.

Conclusion: The adult CB17 mouse LN retains enough structural architecture and cellular content to support the migration of human DC yet lacks signals necessary to support efficient migration into areas of the LN where T and B cells interact with DC. Humanization of CB17 LN prior to injection of human DC appears to draw more DC into central areas of the LN. Monocyte-derived DC obtained from both normal and cancer individuals migrated to the CB17 LN. Using this preclinical model we will be able to determine whether DC from cancer patients migrate as well as those from normal humans and which DC maturation protocols lead to the migration of the greatest numbers of DC to the LN.

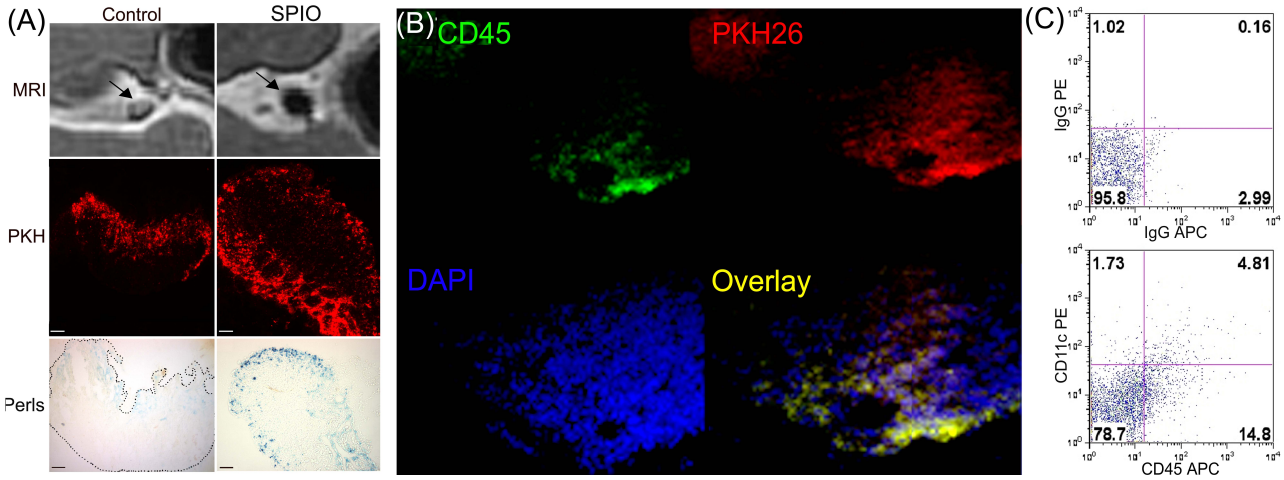

Fig. 1. (A) Images of the left (control) and right (SPIO) CB17 LN (arrows) following the MRI scan (top row), digital fluorescence imaging of PKH positive cells (middle row) and digital bright fileld image of Perl's Prussian blue staining for iron (bottom row). (B) Digital fluorescence imaging of a LN following injection of a footpad with PKH^+ SPIO -labeled to detect human $CD45^+$ (green) and PKH^+ (red) double labeled cells. (C) Flow cytomtery identifying human cells in a LN following the injection of SPIO-lableled, $CFSE^+$ DC into the footpad. Human cells