Longitudinal tracking and quantification of T cells using in vivo 19F MRI

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Abstract

The dynamic trafficking of a specific subset of activated, antigen-specific T cells was studied *in vivo* using ^{19}F MRI in an acute inflammation model for up to 21 days. The perfluoropolyether (PFPE) nanoemulsion was readily taken up by non-phagocytic T cells. T cells inoculated into a localized, acute inflammation mouse model selectively homed to the draining lymph node (DLN). The use of ^{19}F MRI allows for quantification of total ^{19}F signal in the DLN and apparent cell numbers. This technology allows for dynamic monitoring and quantification of cell numbers *in vivo*, without the complications of the large ^{1}H background associated with metal-based contrast agents. These techniques can be adapted to study numerous other cell types.

Introduction

Activated T cells are key effectors of the immune system, and can be either protective or pathogenic. Elucidating the role of T cells in an inflammatory response is of fundamental importance in a broad range of diseases, such as cancer and autoimmunity. The ability to non-invasively visualize and quantify the trafficking of inflammatory T cells is potentially a powerful diagnostic marker and can be useful in the development of new therapeutics. Previously, we developed ^{19}F nanoparticles and labeling methods for activated T cells in the NOD mouse model [1]. In the present study we investigate the trafficking of activated T cells in a murine model of localized inflammation showing a reproducible trafficking of T cells to the DLN.

Methods

$CD4^+$ T cells ($11x10^6$) from DO11.10 transgenic mice [2] were purified, activated *in vitro*, labeled with PFPE nanoparticles [1], and transferred i.p. into recipient BALB/c mice (n=3). Prior to transfer, the mice received ovalbumin in incomplete Freud's adjuvant subcutaneously near the right quadriceps. Mice were imaged longitudinally at day 2, 4, 7, 11 and 21 post-transfer. For MRI, mice were anesthetized with ketamine/xylazine by continuous infusion during the imaging session via an i.p. catheter. Mice were intubated and connected to a mechanical ventilator delivering an O_2/NO_2 mixture. Respiratory-gated imaging was performed using a ^{19}F /^{1}H volume birdcage resonator in an 11.7 T/89 mm vertical-bore micro-imaging system. ^{1}H images were acquired with a 2DFT spin-echo sequence with TR/TE=1200/22 ms and 512×256 image points. ^{19}F acquisitions used a RARE spin-echo sequence with TR/TE=1000/6.4 ms and 64×32 image points. Eight contiguous, 2 mm-thick slices were acquired for both ^{19}F and ^{1}H with a FOV of 5×2.8 cm. The total imaging session lasted ~2 hours. The quantity of apparent labeled cells in regions of interest was calculated directly from the *in vivo* MRI data set, an external ^{19}F reference, and the ^{19}F content per cell, F_c, determined from prior *in vitro* ^{19}F NMR measurements of cell pellets [1].

Results

Labeled cells were visible in the indicated inguinal lymph node, the DLN (Fig.1), and remained detectable for at least 3 weeks. Mesenteric localization was apparent only at the earlier time points (Fig. 1a). By day 11 (Fig. 1b) T cells were only visible in the DLN. Fig. 1c displays the total apparent ^{19}F signal in the DLN over time. Assuming F_c is constant, this is equivalent to $2.1x10^6 \pm 9x10^5$ apparent cells at day 2, dropping to $6x10^5 \pm 2x10^5$ at day 4, and increasing to $3.6 \times 10^6 \pm 9x10^5$ at day 7 post-transfer. These data were validated using detailed conventional flow cytometric cell tracking, functional assays and histological studies.

Discussion

In this study, antigen-specific PFPE labeled T cells were longitudinally tracked and quantified in an acute inflammation model. Neither T cells that were transferred without the concurrent transfer of antigen nor non-antigen specific T cells reached the DLN in appreciable numbers, highlighting the specific nature of the T cell trafficking. The inflammation model can be applied to preclinical studies of therapeutics (e.g., small molecules or recombinant proteins) that modulate T cell trafficking. The *in vivo* ^{19}F platform developed here can readily be extended to other cells types including, for example, immunotherapeutics or stem cells.

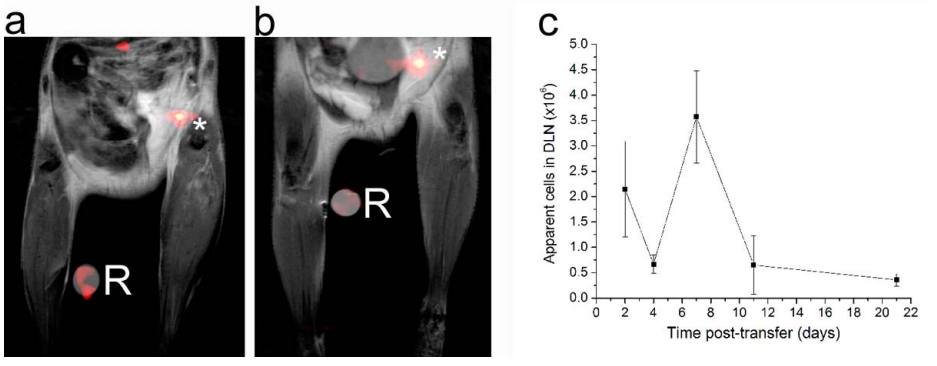

Fig. 1 Representative ^{19}F/^{1}H coronal slices and quantification of total ^{19}F signal in the DLN.
(**a and b**) The MR images are an overlay of the ^{19}F image (false color) and the ^{1}H (grayscale), at day 2 (**a**) and day 11 (**b**) post-transfer. The DLN (asterisk) and external reference (R) are labeled. T cells are visible homing to the DLN. The reference (R) was used for cell number quantification. (**c**) The plot summarizes the quantification results for the 21 day period. Error bars represent standard deviation (n=3).

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References

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