Non invasive in vivo monitoring of dimethyl fumarate treatment in EAE by assessing the glucose metabolism in secondary lymphoid organs

Dimethyl fumarate (DMF) is a small molecular therapeutic approved for the treatment of inflammatory autoimmune diseases like psoriasis and multiple sclerosis (MS), which are dominated by Th1/Th17 cells. As reported, DMF modulates innate and adaptive immune mechanisms by suppressing IL-12/IL-23, inducing IL-10 in antigen presenting cells (APC) and additionally by modulating distinct intracellular pathways [1–3]. Nevertheless, adequate methods for the non-invasive in vivo assessment of the course of disease and the response to treatment are missing. We aimed to evaluate whether T cell activation and anti-inflammatory properties of DMF can be monitored non-invasively in vivo by determining the glucose metabolism in the draining lymph nodes using 2-deoxy-2-[18F]fluoro-D-glucose ([18F]FDG) positron emission tomography (CT), a well-established imaging technique in preclinical research and clinical healthcare. Recently, we investigated the effects of cancer immunotherapy on the [18F]FDG uptake in primary and secondary lymphoid organs, suggesting that altered glucose metabolism is an appropriate way to assess a systemic adaptive immune responses [4]. Here, we correlated the clinical course of EAE in mice with [18F]FDG-PET/CT imaging focusing on the draining lymph nodes and the nondraining (LNs). In agreement with previous reports [1], mice immunized with PLP139-151 peptide developed a severe encephalomyelitis with clinical symptoms starting on day 12 after immunization with an average EAE score of 2.0 ± 0.25 SEM (Fig. 1A). To determine whether quantification of the [18F]FDG-uptake in draining popliteal lymph nodes correlates with the clinical symptoms of EAE, mice were serially imaged with PET/CT on day 3, 7 and 10 after immunization. LNs were identified by fusion of the anatomical CT scans with the functional PET images. On day 7 and 10 after immunization for EAE in the hind food pad, the draining popliteal lymph nodes (pLN) displayed a significantly higher [18F]FDG uptake when compared to healthy mice (p < 0.001; Fig. 1B and C). In line with the enhanced [18F]FDG uptake in the draining pLN, we determined a 17-fold higher expression of Il12b in immune cells of the draining LNs when compared to the non-pathogenic immune response against OVA 323–339 peptide. In accordance with our [18F]FDG-PET/CT imaging results, we determined a 17-fold higher expression of Il12b in immune cells of the draining pLN on day 7 after PLP peptide immunization when compared to OVA peptide (Fig. 1E). Thus, the observed induction of Il12b expression [1] after immunization with PLP and OVA peptide nicely correlates with our in vivo [18F]FDG-PET/CT imaging data [7]. In contrast to the draining pLN, [18F]FDG-PET/CT imaging could not identify any enhanced glucose metabolism ID/g due to PLP immunization in the non-draining iLN at days 3, 5, and 7 after immunization compared to OVA peptide immunization (day 7; PLP 3.7 ± 1.0%; OVA: 2.9 ± 0.7% ID/g) (Fig. 1F).

To decipher between unspecific and autoantigen-specific T cell activation with [18F]FDG-PET/CT imaging in vivo, we immunized mice with PLP139-151 peptide or a non-relevant peptide (OVA 323–339) and quantified the [18F]FDG uptake in the lymph nodes of interest on day 3, 5 and 7 after immunization. On day 7, we detected a significantly higher [18F]FDG uptake in draining pLN of mice immunized with PLP139-151 compared to mice immunized with the irrelevant OVA 323–339 peptide (7.1 ±1.5 %ID/g for PLP and 5.3%±1.8 %ID/g for OVA, respectively; Fig. 1D). These data indicate that the EAE inducing PLP139-151 specific immune response is associated with a more intensely increased glucose metabolism within the draining LNs compared to the non-pathogenic immune response against OVA 323–339 peptide.

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Figure 1. [18F]FDG-PET/CT imaging allows early identification of EAE and provides evidence of specific T-cell activation. (A) SJL mice were immunized for EAE. The result shows mean EAE Score ± SEM from 2 experiments with n = 10. (B and C) Mice were serially imaged on day 3, 7, 10 after immunization. [18F]FDG-PET/CT images from single mice are depicted in (B), pooled data of two experiments from control (n = 5) and EAE immunized mice (n = 4-5) are shown in (C). Box plots in (C, D, and F) show the median with 25th and 75th interquartile range (IQR) and whiskers indicate 1.5 x IQR (*p < 0.05; **p < 0.01). Data in (C, D) were analyzed using Tukey’s post hoc test after One-way ANOVA. (D-F) Mice were immunized with PLP or OVA peptide. The [18F]FDG uptake in pLN (D) and iLN (F) was quantified on day 3, 5, 7 by PET/CT imaging after immunization. Additionally Il12b was determined in pLN on day 7 by qPCR and normalized to Actb and B2m. (E) Bars represent mean ± SEM. (*p < 0.05; Mann-Whitney test). PCR analyses were performed in technical triplicates. Pooled data of three independent experiments from OVA (n = 7) and PLP (n = 7) immunized mice are shown in (D-F).

To evaluate whether non-invasive [18F]FDG-PET/CT imaging is a tool to monitor and quantify anti-inflammatory effects of therapeutics, we treated mice before EAE induction with the clinically approved small molecular drug DMF [1, 2]. In agreement with previous reports, onset of EAE in DMF-treated mice was delayed and mice exhibited only with mild or no symptoms. To study whether oral DMF treatment affects T cell activation in draining or non-draining LNs in vivo, we performed [18F]FDG-PET/CT imaging and determined on day 7 after EAE induction a significantly lower [18F]FDG uptake in the draining pLN of DMF-treated mice (4.1 ±1.3% ID/g) compared to non-treated mice (5.9±1.7% ID/g; Fig. 2A). Ex vivo biodistribution analysis confirmed our noninvasive in vivo [18F]FDG-PET/CT imaging results (Fig. 2B). In agreement to our previous studies [1, 2], qPCR analysis of the respective draining pLN on day 7 after EAE induction yielded a 2-fold lower Il12b expression as a consequence of DMF-treatment (Fig. 2C). In contrast, in vivo [18F]FDG-PET/CT imaging and ex vivo biodistribution analysis exhibited no DMF-treatment associated differences within the nondraining iLN (DMF: 3.6 ± 1.3% ID/g; control: 5.0 ± 2.9% ID/g; Fig. 2D, E).

Interestingly, as a consequence of the systemic effect of DMF treatment, we determined (Fig. 2C) a twofold reduction of Il12b mRNA expression levels in the nondraining iLN compared to untreated animals (Fig. 2F) which was not reflected by the [18F]FDG uptake (Fig. 2D, E).

As to our knowledge, while previous imaging studies on autoimmune diseases focused mainly on the affected inflamed target organs, this is the first study to visualize the systemic immune response after initiation of a Th1/Th17 mediated disease model by monitoring the glucose metabolism in lymph nodes using [18F]FDG PET/CT imaging. Furthermore, we were able to monitor the anti-inflammatory treatment effect of DMF by determination of the glucose metabolism within the draining pLN by non invasive in vivo [18F]FDG-PET/CT imaging.

Preclinical and clinical non-invasive in vivo [18F]FDG-PET/CT is a high sensitive
DMF ameliorates T-cell activation quantified by [18F]FDG uptake and inhibits Il12b production in vivo. (A–F) Mice received DMF or control water, immunized for EAE, and serially imaged on days 3, 5, and 7. [18F]FDG uptake in pLN and iLN was quantified by PET/CT imaging and biodistribution. Pooled [18F]FDG-PET/CT (A, D) and biodistribution (B, E) data from three independent experiments are shown (DMF n = 8; control n = 10). pLN and iLN were simultaneously isolated and Il12b was determined by qPCR and normalized to Actb and B2m. (C, F) PCR analyses were performed in technical triplicates. Pooled data from DMF (n = 8) and control (n = 10) mice from three independent experiments Box plots show the median with 25th and 75th interquartile range (IQR) and whiskers indicate 1.5 x IQR (P<0.05). Bars represent the mean ± SEM (P<0.05) Data in (A to E) were analyzed using Tukey’s post hoc test after One-way ANOVA.
Data availability statement: Data available on request from the authors

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CFA: Complete Freund’s adjuvant  ·  dLNs: draining lymph nodes  ·  DMF: Dimethyl fumarate  ·  EAE: experimental autoimmune encephalomyelitis  ·  [18F]FDG: 2-deoxy-2-[18F]fluoro-D-glucose  ·  iLN: inguinal lymph nodes  ·  LNs: lymph nodes  ·  MRI: magnetic resonance imaging  ·  MS: multiple sclerosis  ·  PET/CT: positron emission tomography/computed tomography  ·  pLN: popliteal lymph nodes  ·  PT: Pertussis toxin

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