

Letter to the Editor

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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- ^{18}F fluoro-D-glucose (^{18}F FDG) positron emission tomography (PET)/computed

tomography (CT), a well-established imaging technique in preclinical research and clinical healthcare. Recently, we investigated the effects of cancer immunotherapy on the ^{18}F 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 ^{18}F FDG-PET/CT imaging focusing on the draining lymph nodes and the nondraining (LNs). In agreement with previous reports [1], mice immunized with PLP_{139–151} 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 ^{18}F 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 ^{18}F FDG uptake when compared to healthy mice ($p < 0.001$; Fig. 1B and C). In line with the enhanced ^{18}F FDG uptake in the draining pLN, we determined a significantly enhanced glucose metabolism within the nondraining inguinal lymph nodes (iLN) 7 and 10 days after immunization. These results are in agreement with previous reports demonstrating that ^{18}F FDG uptake by activated immune cells or proliferating tumor cells correlate with the course of disease in various autoimmune or tumor diseases [5–7].

To decipher between unspecific and autoantigen-specific T cell activation with ^{18}F FDG-PET/CT imaging *in vivo*, we immunized mice with PLP_{139–151} peptide or a non-relevant peptide (OVA_{323–339}) and quantified the ^{18}F FDG uptake in the lymph nodes of interest on day 3, 5 and 7 after immunization. On day 7, we detected a significantly higher ^{18}F FDG uptake in draining pLN of mice immunized with PLP_{139–151} 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 PLP_{139–151} specific immune response is associated with a more intensely increased glucose metabolism within the draining LNs when compared to the non-pathogenic immune response against OVA_{323–339} peptide. In accordance with our ^{18}F 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* ^{18}F FDG-PET/CT imaging data [7]. In contrast to the draining pLN, ^{18}F 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). Our results demonstrate that ^{18}F FDG-PET/CT imaging focusing on dLNs can be employed to distinguish between specific and non-specific T cell activation in inflammatory autoimmune diseases.

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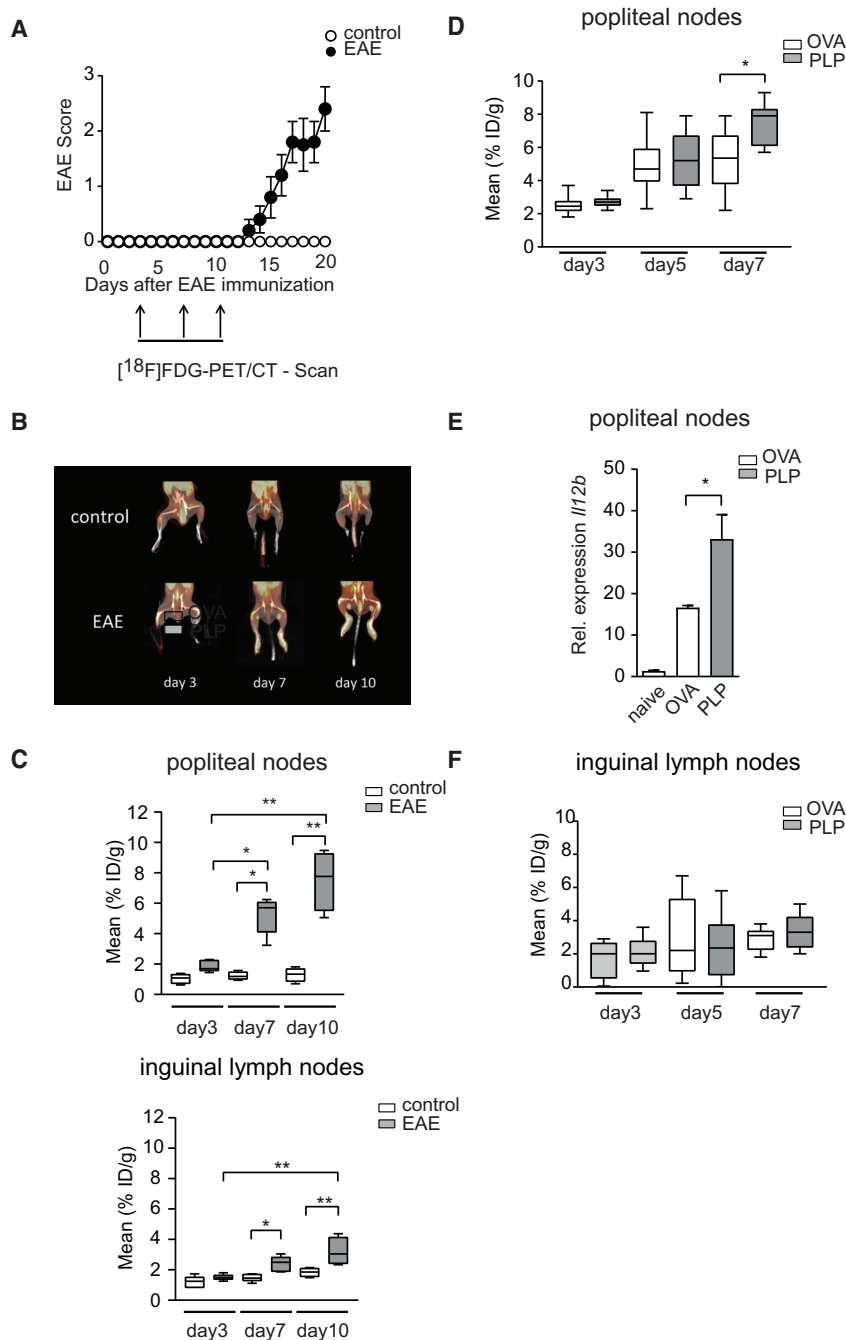


Figure 1. $[^{18}\text{F}]\text{FDG}$ -PET/CT imaging allows early identification of EAE and provides evidence of specific T-cell activation. (A) SJL mice were immunized for EAE. The result show mean EAE Score $\pm$ SEM from 2 experiments with $n = 10$. (B and C) Mice were serially imaged on day 3, 7, 10 after immunization. $[^{18}\text{F}]\text{FDG}$ -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 $1.5 \times \text{IQR}$ (* $p < 0.05$; ** $p < 0.01$). 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 $[^{18}\text{F}]\text{FDG}$ 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 analysis and normalized to *Actb* and *B2m*. (E) Bars represent mean $\pm$ 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 $[^{18}\text{F}]\text{FDG}$ -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 $[^{18}\text{F}]\text{FDG}$ -PET/CT imaging and determined on day 7 after EAE induction a significantly lower $[^{18}\text{F}]\text{FDG}$ uptake in the draining pLN of DMF-treated mice ($4.1 \pm 1.3\%$ ID/g) compared to non-treated mice ($5.9 \pm 1.7\%$ ID/g; Fig. 2A). *Ex vivo* biodistribution analysis confirmed our noninvasive *in vivo* $[^{18}\text{F}]\text{FDG}$ -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* $[^{18}\text{F}]\text{FDG}$ -PET/CT imaging and *ex vivo* biodistribution analysis exhibited no DMF-treatment associated differences within the nondraining iLN (DMF: $3.6 \pm 1.3\%$ ID/g; control: $5.0 \pm 2.9\%$ 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 $[^{18}\text{F}]\text{FDG}$ 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 $[^{18}\text{F}]\text{FDG}$ 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* $[^{18}\text{F}]\text{FDG}$ -PET/CT imaging.

Preclinical and clinical non-invasive *in vivo* $[^{18}\text{F}]\text{FDG}$ -PET/CT is a high sensitive

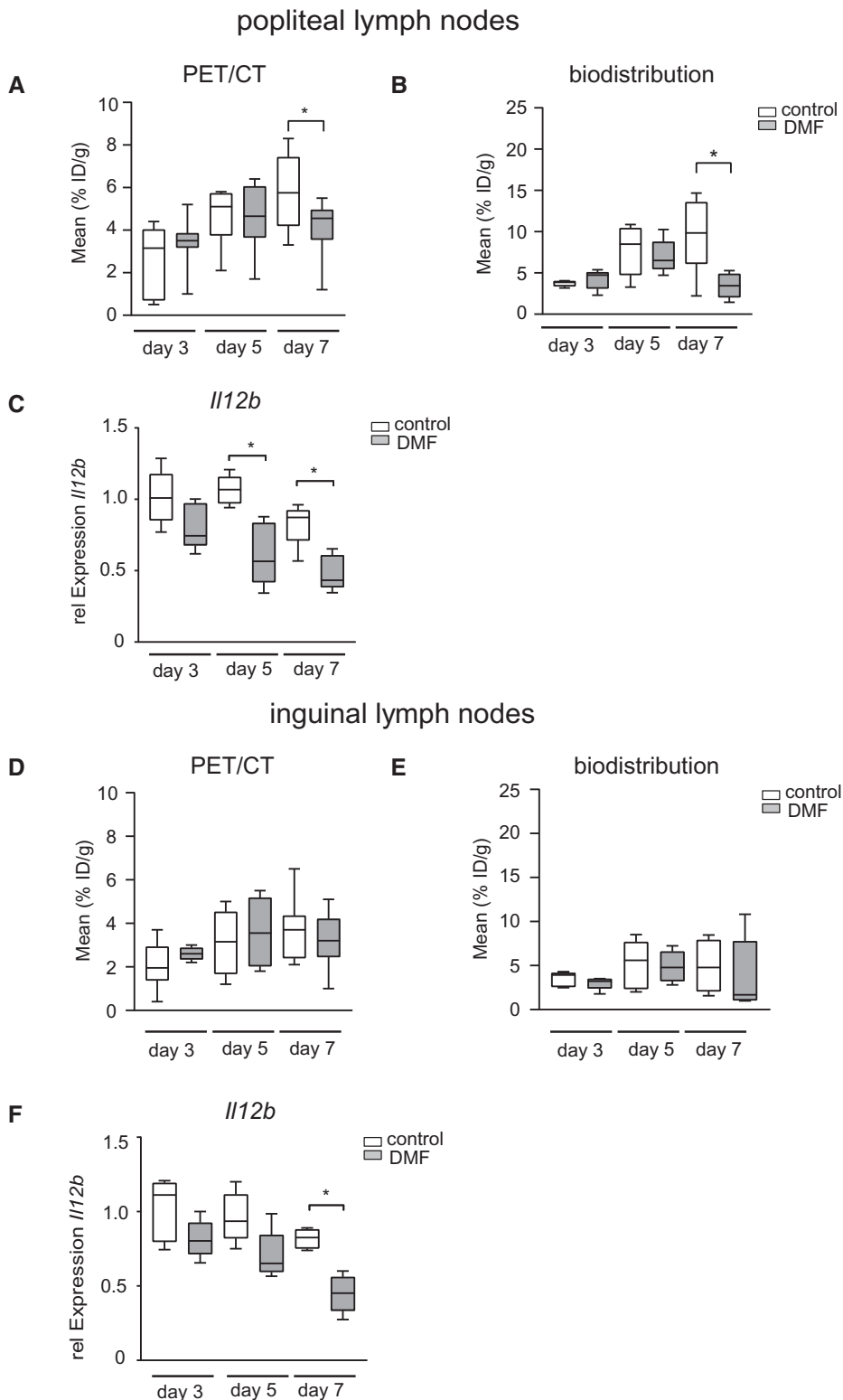


Figure 2. DMF ameliorates T-cell activation quantified by [^{18}F]FDG uptake and inhibits *Il12b* production in vivo. (A–F) Mice received DMF or control water, immunized for EAE, and serially imaged on days 3, 5, and 7. [^{18}F]FDG uptake in pLN and iLN was quantified by PET/CT imaging and biodistribution. Pooled [^{18}F]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 are shown. Box plots show the median with 25th and 75th interquartile range (IQR) and whiskers indicate $1.5 \times \text{IQR}$ (* $P < 0.05$). Bars represent the mean $\pm$ SEM (* $P < 0.05$). Data in (A to E) were analyzed using Tukey's post hoc test after One-way ANOVA.

functional diagnostic imaging modality of increasing importance not only in oncology but also in autoimmune diseases facilitating longitudinal monitoring of novel innovative immunotherapeutic treatment

approaches.[8, 9] of autoimmune disorders and malignancies [10].

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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 tomog-

raphy · **pLN:** popliteal lymph nodes · **PT:** Pertussis toxin

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