Dimethyl fumarate suppresses neurodegeneration through reduction of M1 macrophages-induced A1 reactive astrocytes and complement C3 deposition

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ABSTRACT:

Dimethyl fumarate (DMF) is an oral agent for relapsing-remitting multiple sclerosis (RRMS). In this study, we investigated the therapeutic mechanism of DMF using experimental autoimmune encephalomyelitis (EAE). DMF treatment decreased the proliferation of T cells and the production of IL-17A and GM-CSF. DMF treatment also decreased the infiltration of macrophages into the central nervous system (CNS), and reduced the ratio of M1 vs M2 macrophages. Furthermore, DMF-treatment suppressed the deposition of complement C3 (C3) and development of reactive A1 astrocytes. The decrease in M1 macrophages, reactive A1 astrocytes, and C3 deposition in the CNS resulted in reduction of demyelination and axonal loss. This study suggests that the beneficial effect of DMF involves the suppression of M1 macrophages, reactive A1 astrocytes, and deposition of C3 in the CNS.

INTRODUCTION:

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS). Infiltration of pathogenic immune cells into the CNS causes disease initiation and progression in MS. Among FDA approved disease modifying agents for relapsing-remitting forms of MS (RRMS), dimethyl fumarate (DMF) is an oral drug with neuroprotective and immunomodulatory effects. Although not completely understood, DMF's therapeutic effect may involve both Nrf2 dependent and independent pathways. DMF confers neuroprotection during neuroinflammation by induction of Nrf2 anti-oxidative pathway in glial cells and neurons. Demyelination and axonal loss are hallmark of MS, and glia cells including microglia and astrocytes play a pivotal role in neurodegeneration. Especially, development of M1 macrophages/microglia and reactive A1 astrocytes is highly involved in disease progression. Proinflammatory cytokines produced by infiltrated immune cells and TLR ligands promote the development of M1 macrophages and reactive A1 astrocytes. Importantly, C3 produced in the glia cells plays a pathogenic role in the development of M1 macrophages and reactive A1 astrocytes. In this study, we investigated the effect of DMF-treatment on the development of M1/M2 macrophages, development of reactive A1 astrocytes, and C3 deposition in the CNS.

RESULTS:

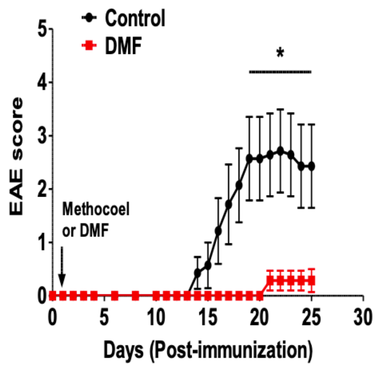

Figure 1. Effect of DMF on MOG-EAE. EAE was induced in C57BL/6 mice by immunization with MOG35-55/CFA and injection of pertussis toxin, as described in Materials and Methods. The immunized mice were treated orally with DMF (n = 7) at 100 mg/kg or 0.8% methocel as a control (n = 7) every day from 1 day post-immunization until the end of the experiment. Data shown is the mean clinical score ± SEM. *P -value <0.05 compared to a control.

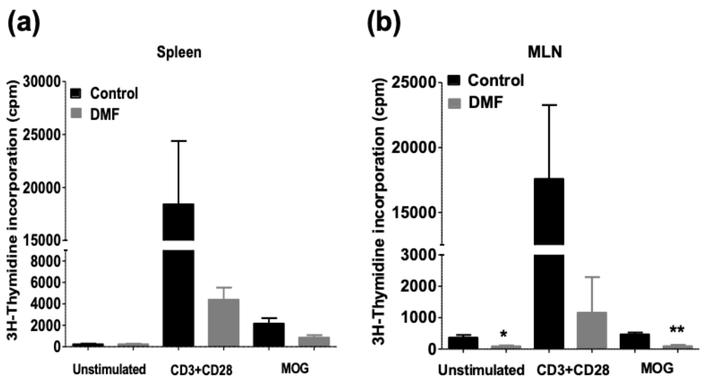

Figure 2. Effect of DMF on T cell proliferation in MOG-EAE mice. Mice were orally treated with DMF (100 mg/kg) or 0.8% methocoel from day 1 to day 25 post-immunization. Cells isolated from the (a) spleen (n=3-4), and (b) MLNs (n=3-4), were cultured with CD3 mAb and CD28mAb or MOG35-55 for three days and were then examined for proliferation by [^{3}H]-thymidine uptake. *P<0.05, **P<0.01.

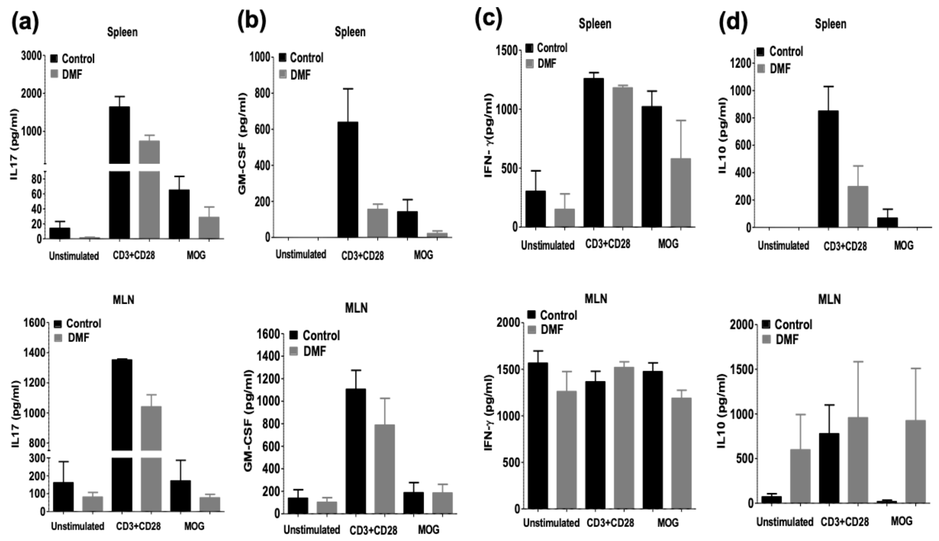

Figure 3. Effect of DMF on cytokine production in the periphery of MOG-EAE mice. Mice were orally treated with DMF (100 mg/kg) or 0.8% methocoel from day 1 to day 25 post-immunization. The cells isolated from spleen (n=3-4) and MLNs (n=3-4), were cultured with CD3 mAb and CD28 mAb or MOG35-55 for three days. Production of IL-17 (a), GM-CSF (b), IFN-γ (c), and IL-10 (d) was measured by ELISA.

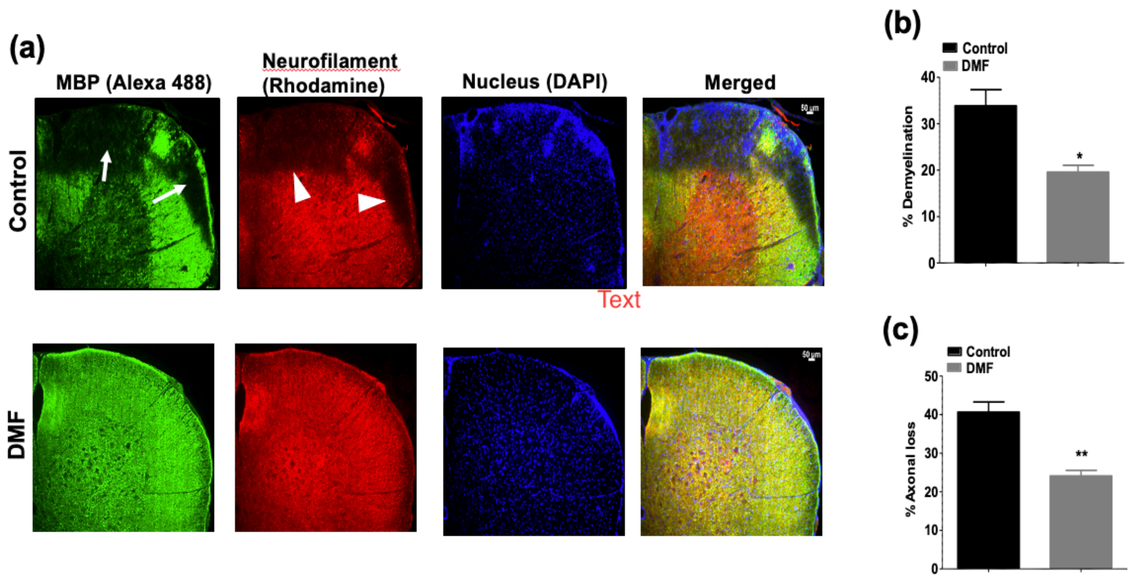

Figure 4. Effect of DMF treatment on demyelination and axonal loss. (a) Mice were sacrificed 25 days after immunization, and demyelination and axonal loss were examined by immunohistology. Demyelination and axonal loss were examined by staining with anti-myelin basic protein (MBP) and anti-neurofilament (NF) Abs, respectively. Cell nuclei were counterstained with DAPI. Areas of demyelination and axonal lossare shown by white arrows and arrow heads, respectively. Demyelination (b) and axonal loss (c) were estimated by measuring the MBP+, NF+, and total tissue areas. n=3-4. *P<0.05, **P<0.01 compared to control. Scale=50 μm.

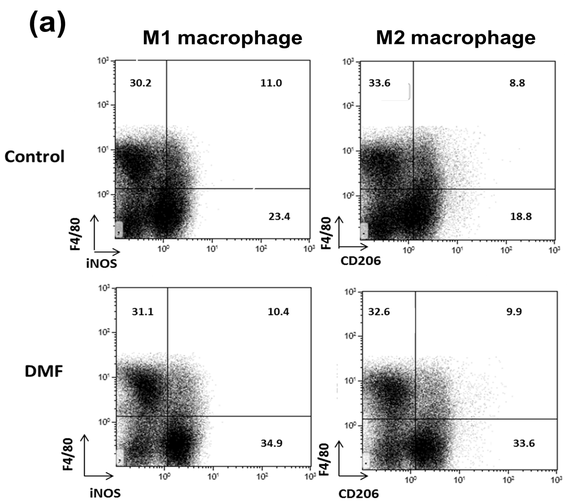

Figure 5. Effect of DMF treatment on M1 and M2 macrophage differentiation in spleen. (a) Differentiation of M1 and M2 macrophages was analyzed by staining the splenocytes with F4/80, iNOS, and CD206 antibodies. F4/80+iNOS+ (M1 macrophage) and F4/80+CD206+ (M2 macrophage) populations were analyzed. Representative flow cytometry data is shown. (b) Quantification of M1 and M2 macrophage population in the spleen.

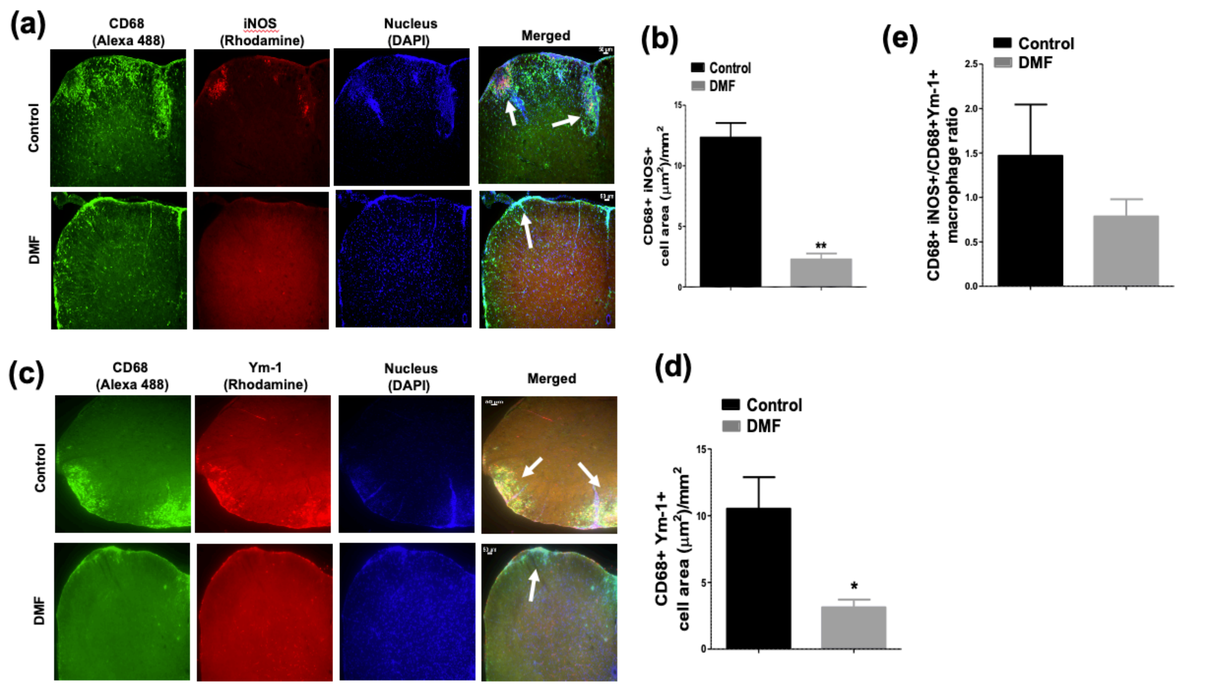

Figure 6. Effect of DMF treatment on infiltration of M1 and M2 macrophages into the spinal cord. Mice were sacrificed 25 days after immunization, and infiltration of M1 and M2 macrophages were examined by immunohistology. (a) Effect of DMF treatment on M1 macrophage in the spinal cord. M1 macrophages were determined by co-staining with anti-iNOS and anti-CD68 Abs. Cell nuclei were counterstained with DAPI. An area of M1 macrophage infiltration is shown by white arrows. (b) Effect of DMF treatment on M2 macrophages in the spinal cord. Infiltration of M2 macrophages was examined by co-staining with anti-Ym-1 and anti-CD68 Abs. (c) Infiltrated area of M1 macrophages was quantified as described in material and method. (d) Infiltrated area of M2 macrophage is shown by white arrows. (e) Effect of DMF treatment on M1/M2 macrophage ratio in the spinal cord. M1/M2 macrophage ratio was calculated as the ratio of CD68+iNOS+ to CD68+Ym-1+ area. n=3-5. *P<0.05, **P<0.01 compared to a control. Scale=50 μm.

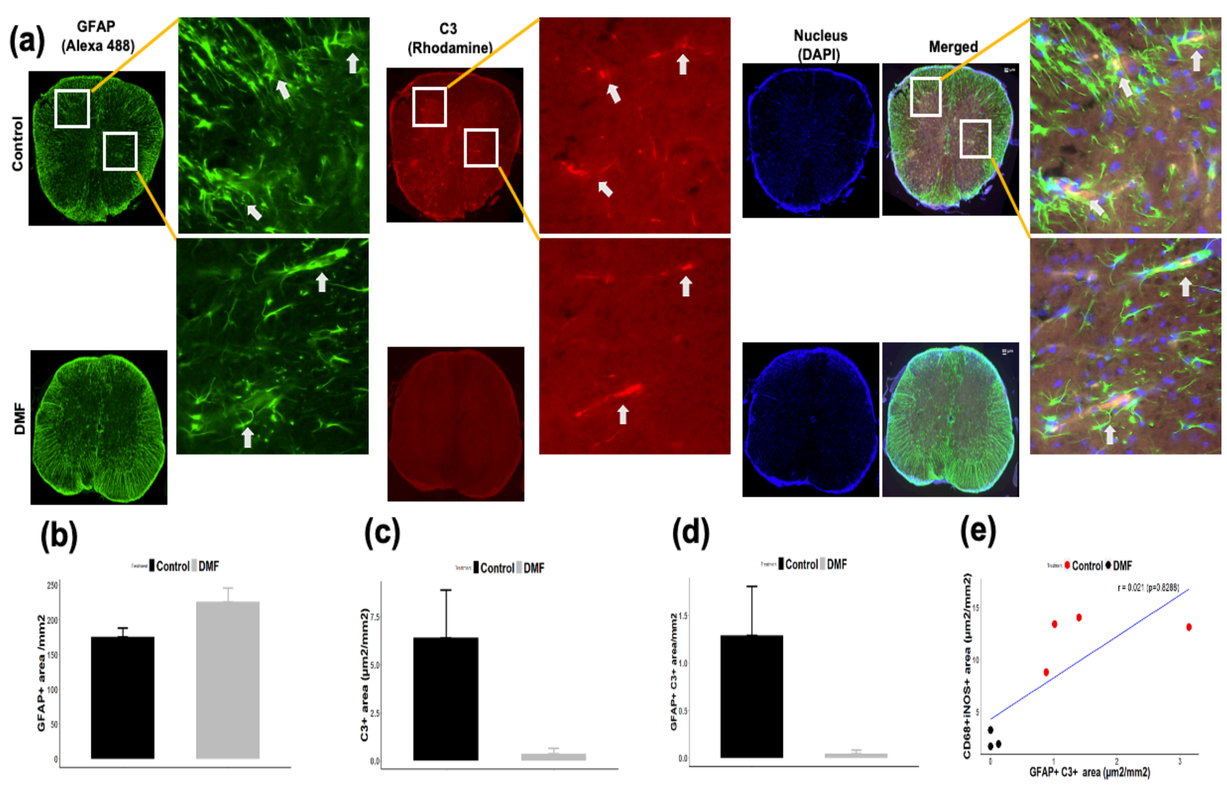

Figure 7. Effect of DMF treatment on deposition of C3 and development of reactive A1 astrocytes in the CNS (a) Mice were euthanized 25 days after immunization, and spinal cords were co-stained with GFAP and C3 mAbs. Cell nuclei were counterstained with DAPI. GFAP$^+$ astrocytes (b), C3 deposition (c) and GFAP$^+$C3$^+$ reactive A1 astrocytes (d) were quantified as described in Material and Method. (e) The correlation between M1 macrophages and reactive A1 astrocytes is presented. Scale=50 μm.

CONCLUSIONS:

1. DMF treatment decreased the proliferation of T cells accompanied by reduced production of IL-17 and GM-CSF.
2. DMF treatment also significantly decreased the infiltration of macrophages, and reduced the M1 / M2 macrophage ratio in the CNS.
3. DMF-treatment suppressed the deposition of complement C3 (C3) and development of reactive A1 astrocytes.
4. The decrease in M1 macrophages, reactive A1 astrocytes, and C3 deposition in the CNS resulted in the reduction of demyelination and axonal loss.
5. The beneficial effect of DMF may involve the suppression of the development of pathogenic Th17 and Th-GMCSF cells in the periphery, and reduction in proinflammatory macrophages and astrocytes in the CNS.

ACKNOWLEDGEMENT

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COMPETING INTERESTS

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