



3H-1, 2-Dithiole-3-Thione (D3T) as a Novel Therapeutic Agent for the Treatment of Experimental Autoimmune Encephalomyelitis

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Disclosure Information

- All authors declare that there are no conflicts of interest.



Learning Objectives

- Examine the potential beneficial effect of D3T in the treatment of EAE, an animal model of MS.
- Investigate the molecular mechanisms underlying the protective effect of D3T in EAE.



Multiple Sclerosis (MS)

- A chronic autoimmune, inflammatory neurological disease of central nervous system (CNS).
 - More than 2.3 million people affected by MS worldwide.
- National Multiple Sclerosis Society website.
- Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model for MS.

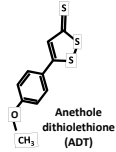
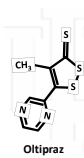
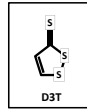




3H-1,2-Dithiole-3-Thione (D3T)

- D3T, a natural compound, can be found in cruciferous vegetables.
- D3T is the parent compound of dithiolethiones, which is a well-known class of cancer chemopreventive agents.

- Mol Cancer Ther (2008)



- D3T provides antioxidant activity through Nrf2 pathway.
- Nrf2 pathway has been reported to provide anti-inflammatory effects. (Nrf2 / HO-1 / NF-κB pathway)

- Oncol Res (1997); Cell Mol Neurobiol (2008); Exp Bio Med (2008)
- J Pharmacol Exp (2006); J Exp Med (2011); J Immunol (2014)



Hypothesis

The effect of D3T in the autoimmune inflammatory disease MS/EAE is unknown

D3T → Nrf2 → anti-inflammation

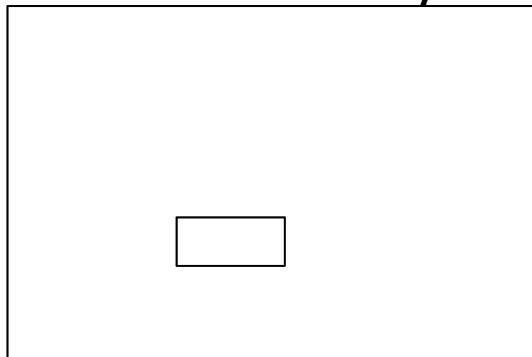
To investigate whether D3T would provide a beneficial effect in EAE through its anti-inflammatory property



D3T Reduces EAE Severity

C57BL/6 female
s.c. MOG immunization
i.p. D3T 10mg/kg
Every day starting from day 1

30d
Stop Treatment
30d
Clinical scores

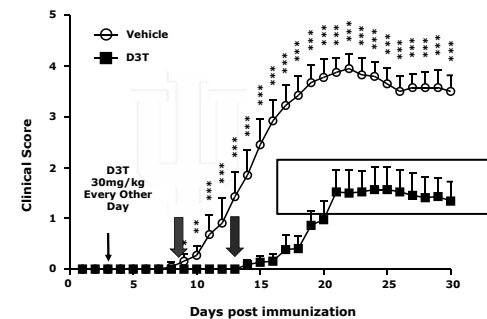


Score	Clinical observations
0	No obvious changes.
1	Limp tail.
2	Limp tail and weakness of hind legs.
3	Limp tail and partial paralysis of hind legs.
4	Limp tail, complete hind leg and partial front leg paralysis.
5	Mouse is found dead due to paralysis.



D3T Reduces EAE Severity

C57BL/6 female
i.p. D3T 30mg/kg
Every other day starting from day 3
30d
Clinical scores





D3T Inhibits EAE Progression

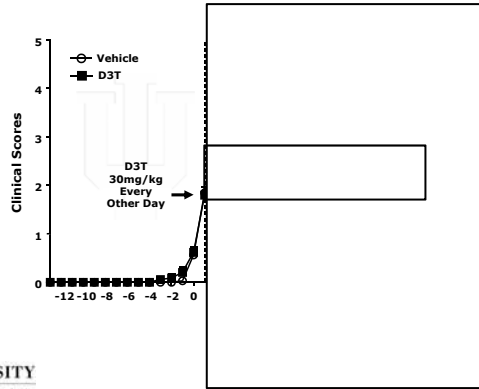
EAE mice
Disease score 1.5-2



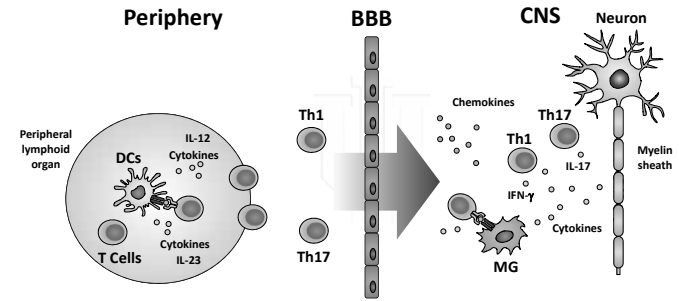
i.p. D3T 30mg/kg
Every other day

20d

Clinical scores



Pathogenesis of MS/EAE

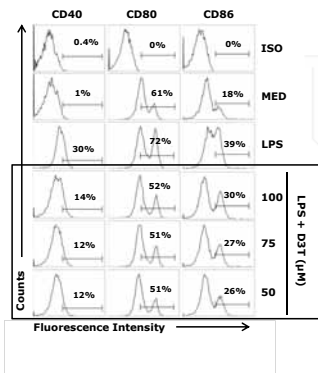


DCs : dendritic cells
MG : microglia
BBB : blood-brain barrier

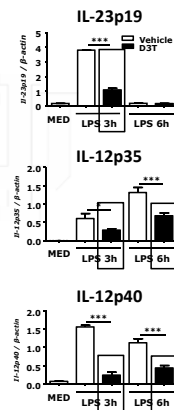


D3T Inhibits DC Activation

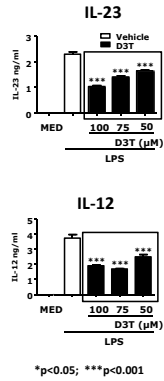
BM-DCs / FACS



BM-DCs / mRNA



BM-DCs / Protein



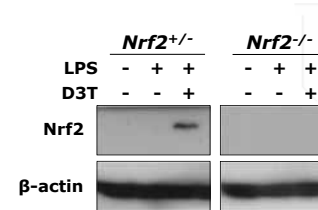
*p<0.05; ***p<0.001



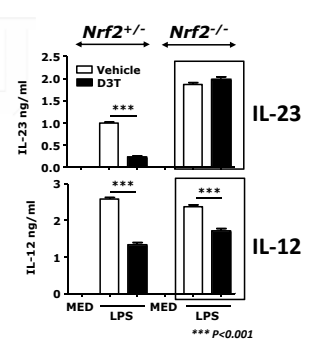
D3T Inhibits DC Activation

Nrf2 pathway \rightarrow anti-inflammation

BM-DCs / WB



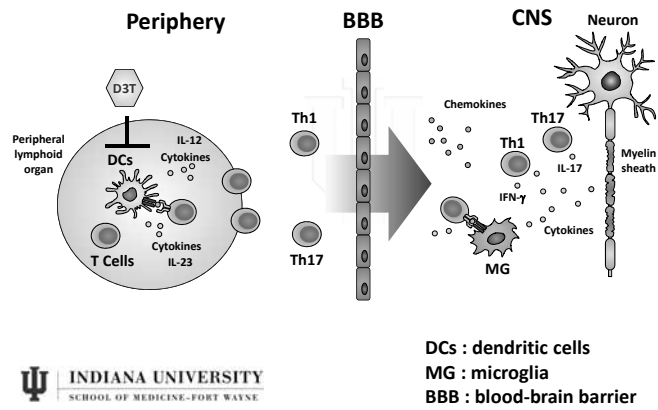
BM-DCs / ELISA



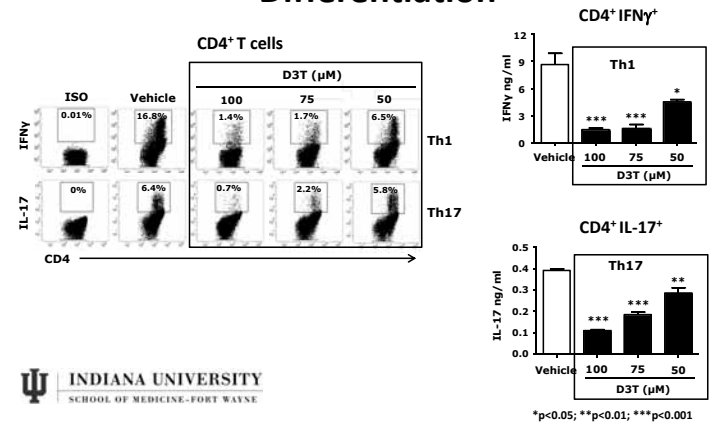
***P<0.001



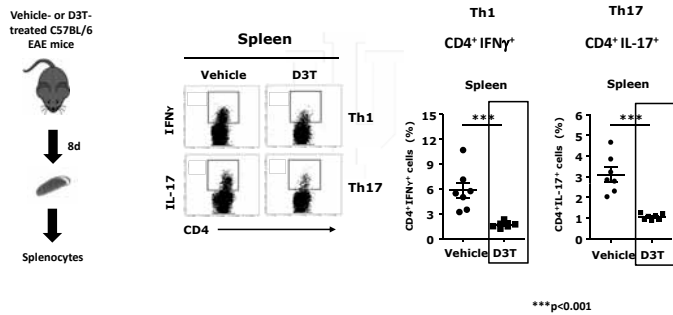
Pathogenesis of MS/EAE



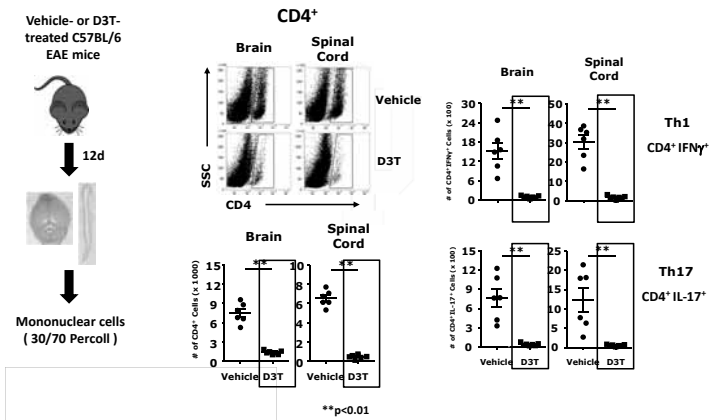
D3T Inhibits Th1 and Th17 Differentiation



D3T Reduces Peripheral Th1/Th17 Differentiation in EAE

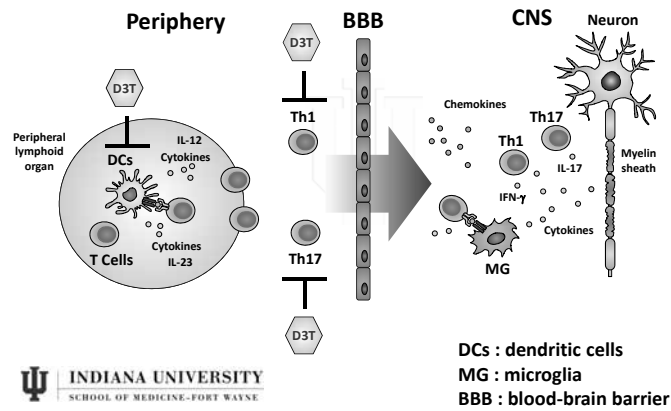


D3T Decreases CNS Th1/Th17 Infiltration in EAE

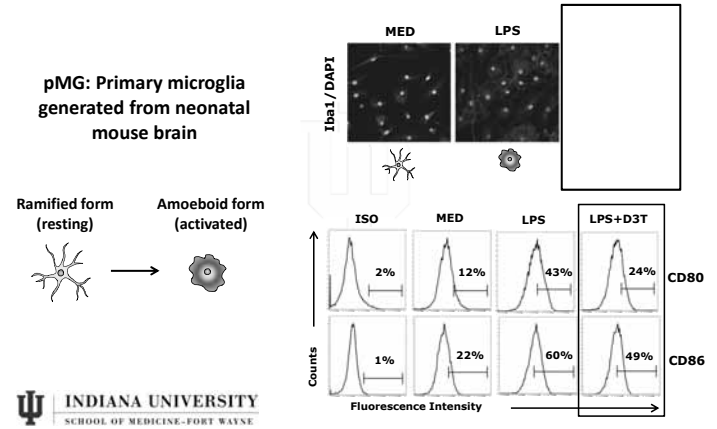




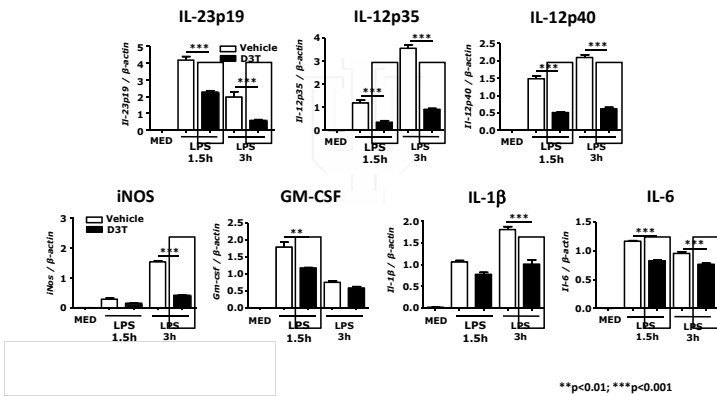
Pathogenesis of MS/EAE



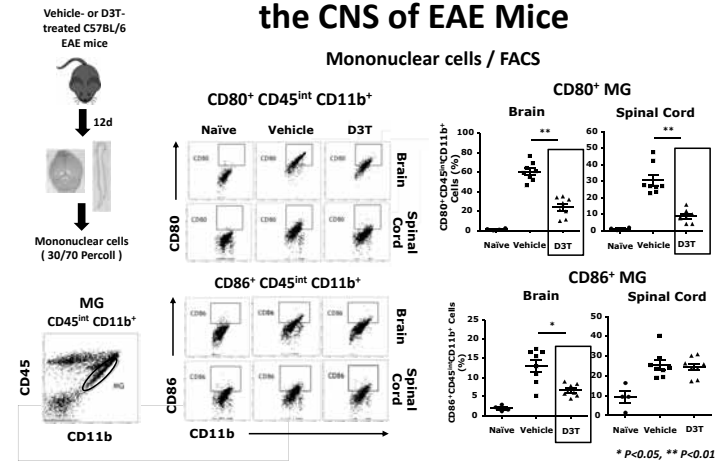
D3T Inhibits MG Activation



D3T Inhibits MG Activation



D3T Suppresses MG Activation in the CNS of EAE Mice





D3T Reduces Inflammatory Cytokine Expression in the CNS of EAE Mice

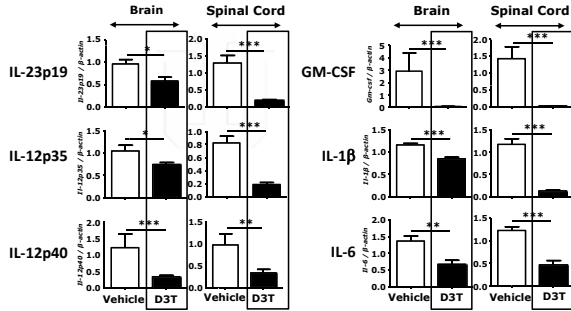
Vehicle- or D3T-treated C57BL/6 EAE mice



12d



Q-PCR

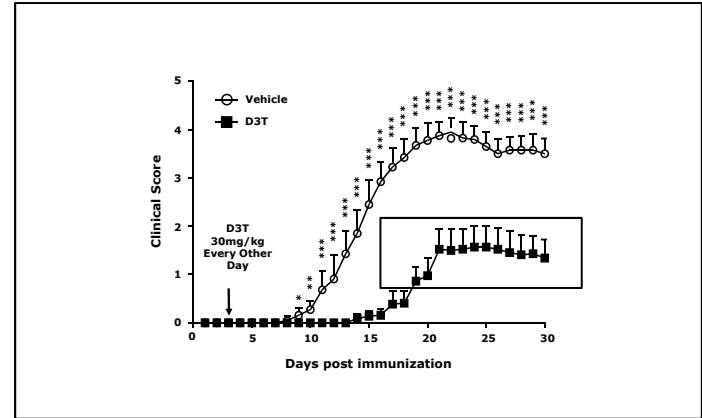


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Conclusion



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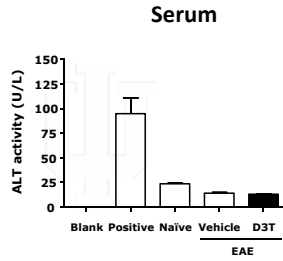
Vehicle- or D3T-treated C57BL/6 EAE mice



i.p. D3T 30mg/kg
Every other day
starting from day 3

30d

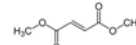
Serum



ALT: alanine transaminase



Dimethyl fumarate (DMF)



C₆H₈O₄

MW : 144.127 g/mol

3H-1, 2-dithiole-3-thione (D3T)

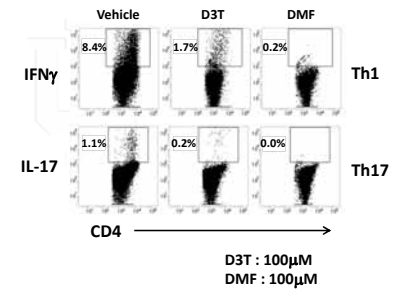


C₃H₂S₃

MW : 134.243 g/mol

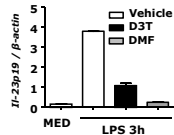
CD4⁺ T cells / FACS

CD4⁺ T cells

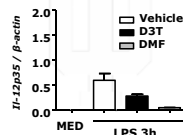


BM-DCs / Q-PCR

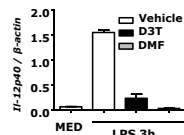
IL-23p19



IL-12p35



IL-12p40



D3T : 100µM
DMF : 100µM
LPS : 100ng/ml



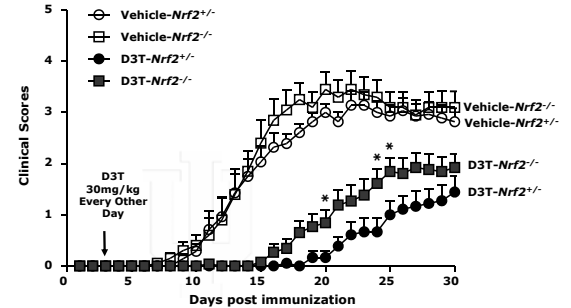
Nrf2^{-/-}, *Nrf2*^{+/-} female



i.p. D3T 30mg/kg
Every other day
starting from day 3

30d

Clinical scores



	<i>Nrf2</i> ^{+/-}		<i>Nrf2</i> ^{-/-}	
	Vehicle	D3T (30mg/kg)	Vehicle	D3T (30mg/kg)
Incidence	14/14	9/9	10/10	13/13
Mortality	1/14	0/9	0/10	0/13
Maximum score	3.9±0.2	1.5±0.3	3.7±0.4	2.1±0.3
Cumulative score	50.1±2.3	9.9±2.5	55.6±5.5	19.7±3.4*

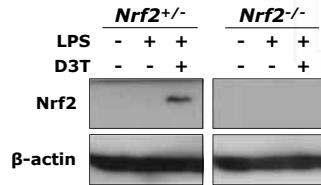
* P<0.05



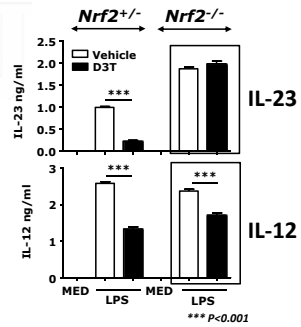
D3T Inhibits DC Activation

Nrf2 pathway → anti-inflammation

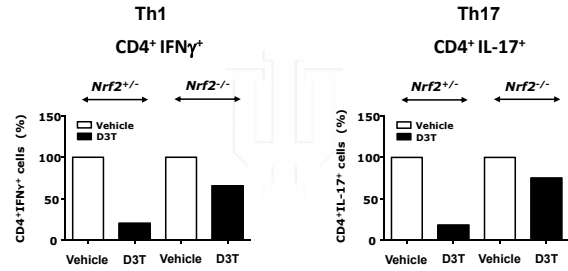
BMDCs / WB



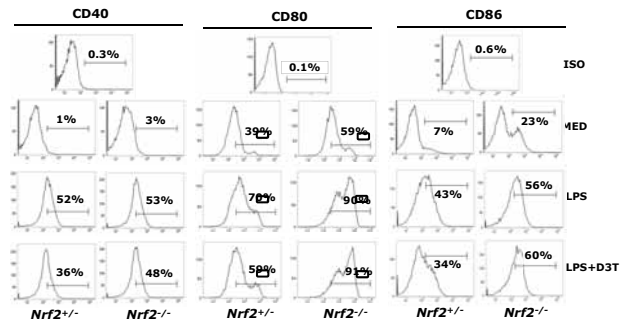
BMDCs / ELISA



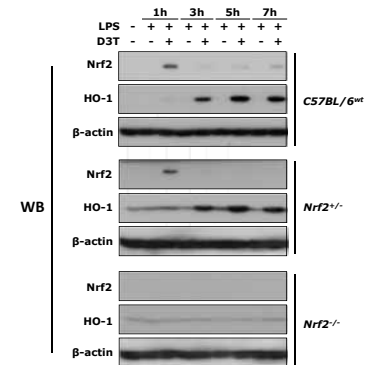
CD4⁺ T cells / FACS



Nrf2^{+/-} & *Nrf2*^{-/-} BM-DCs / FACS

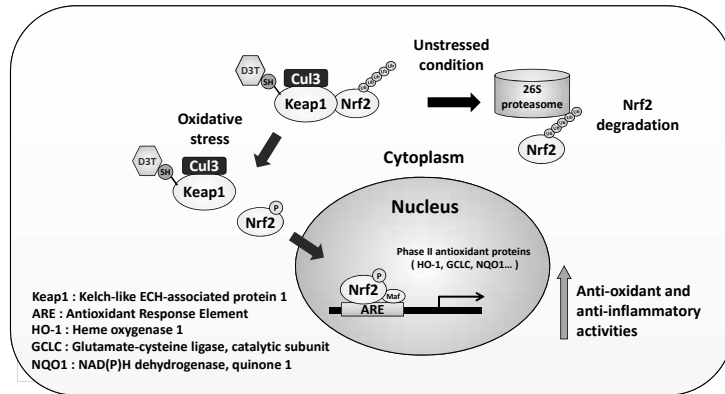


BM-DCs WB

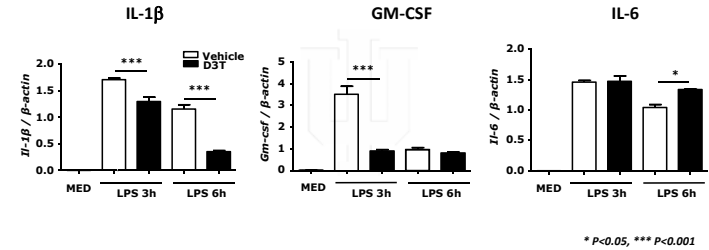


Nrf2 Pathway

- Nuclear factor E2-related factor 2 (Nrf2) is a basic leucine zipper transcription factor.



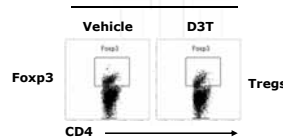
BM-DCs / Q-PCR



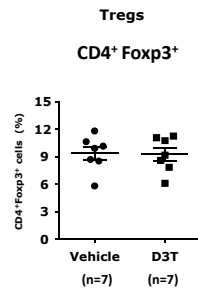
Vehicle- or D3T-treated C57BL/6 EAE mice



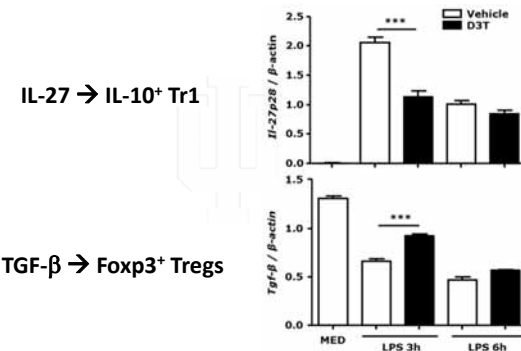
Splenocytes / FACS



Splenocytes / FACS

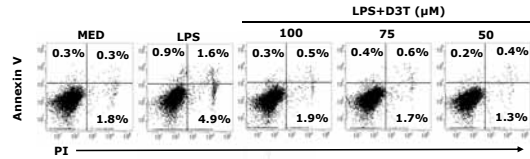


BM-DCs / Q-PCR

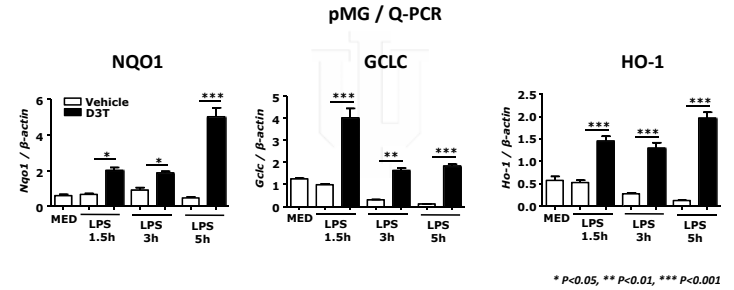




BM-DCs / FACS



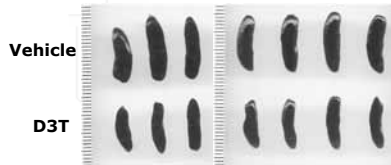
D3T Promotes Phase II Anti-oxidant Enzyme Induction in MG



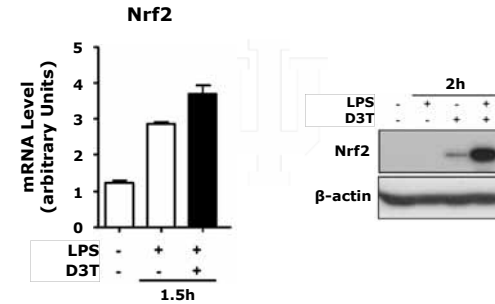
Vehicle- or D3T-treated C57BL/6 EAE mice



Spleens



BV2 Cells





D3T Promotes Phase II Anti-oxidant Enzyme Induction in MG

