Blockade of Bone Morphogenetic Protein-2/4 Induces Oligodendrogenesis and Remyelination in Inflammatory and Toxic Demyelinating Disorders

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BACKGROUND

Oligodendrocyte precursor cells (OPCs) are present in demyelinated lesions of multiple sclerosis (MS). However, their differentiation into functional oligodendrocytes is mostly insufficient, and most lesions evolve into nonfunctional astroglial scars. Blockade of bone Morphogenetic protein (BMP) signaling was found to induce the differentiation of OPCs into myelin-producing oligodendrocytes.

OBJECTIVE

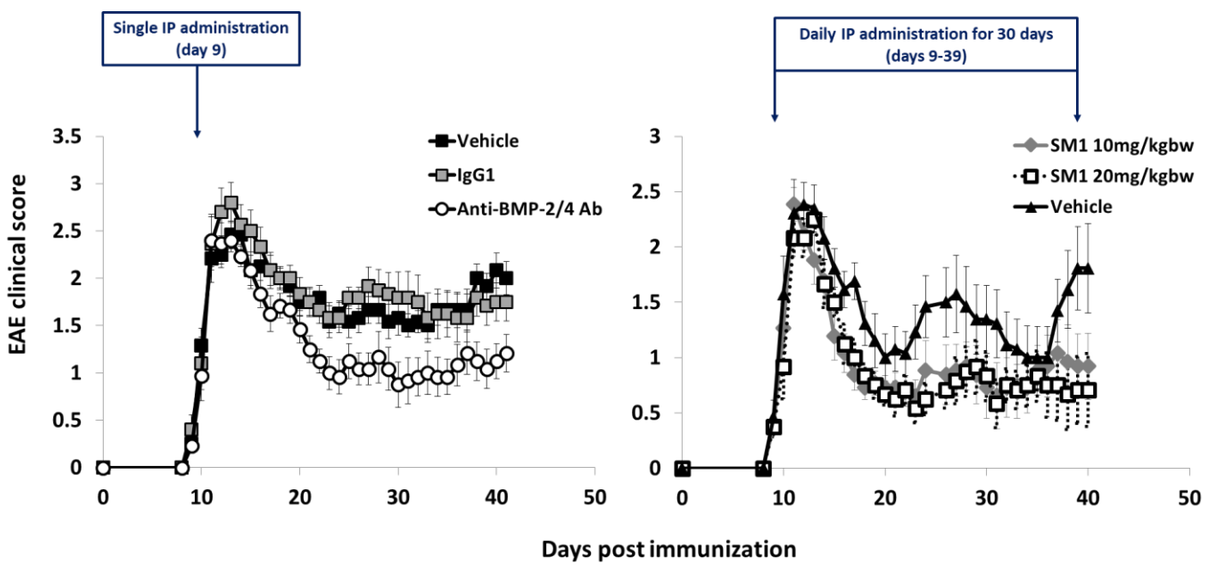
To examine the effect of BMP-2/4 signaling blockade in both the inflammatory model of relapsing experimental autoimmune encephalomyelitis (R-EAE) and the cuprizone toxic model of demyelination in mice.

METHODS

BMP-2/4 signaling was inhibited either by treatment with anti-BMP-2/4 neutralizing mAb (single injection, IV), or by a novel small molecule (SM), that we have discovered *via* high throughput screening (HTS) for BMP-2/4 inhibition in ATDC5 cells bioassay (IP and gavage administration, treatment for 30 days).

RESULTS

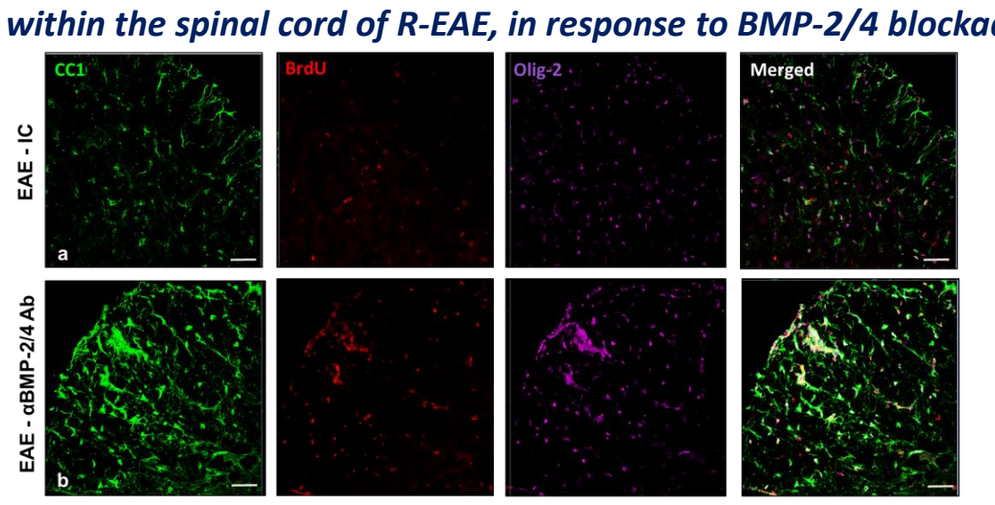
Treatment with anti-BMP-2/4 mAb, or with SM1, ameliorates R-EAE

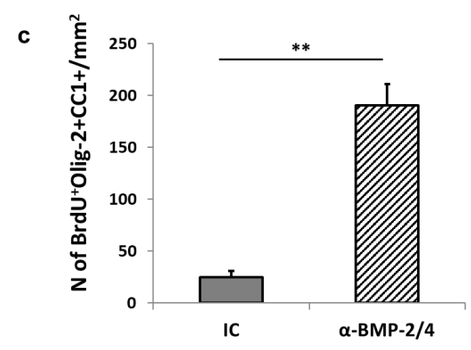


	Vehicle	IgG1	Anti- BMP-2/4 Ab
Incidence (score ≥ 1)	100.0%	100%	100%
Disease onset (days)	9.5 ± 0.2	9.5 ± 0.2	9.6 ± 0.2
Maximum score	3.3 ± 0.3	3.4 ± 0.2	2.8 ± 0.2
Cumulative score	58.4 ± 2.8	57.7 ± 4.3	42.9 ± 4.8*

	Vehicle	SM1 10mg/KgBW	SM1 20mg/KgBW
Incidence (score ≥ 1)	100%	100.0%	100.0%
Disease onset (days)	9.5 ± 0.2	9.4 ± 0.2	9.5 ± 0.2
Maximum score	3.0 ± 0.2	2.6 ± 0.2	2.5 ± 0.1
Cumulative score	44.2 ± 5.8	30.6 ± 4.2	29.0 ± 3.6*

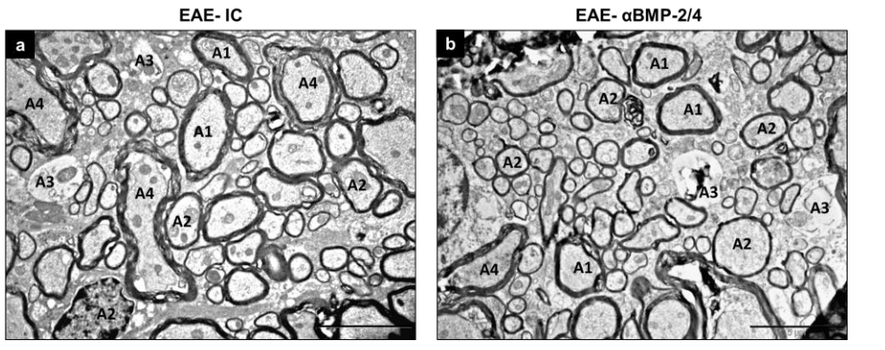
Elevated numbers of de novo BrdU+Olig-2+CC1+ mature oligodendrocytes within the spinal cord of R-EAE, in response to BMP-2/4 blockade

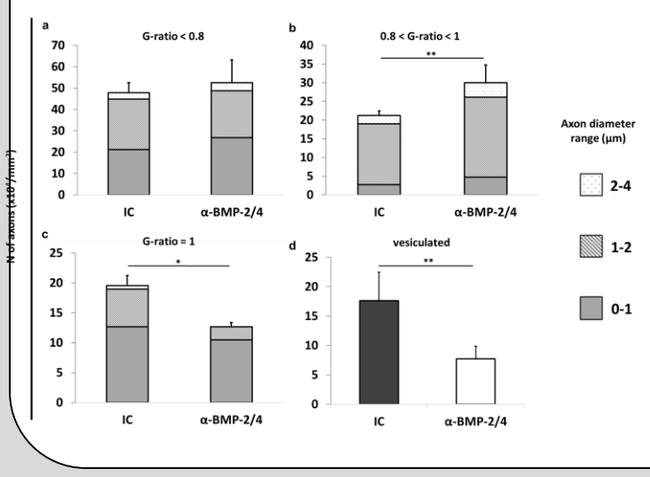




In order to detect post- treatment differentiated cells, three mice in each group were daily IP injected with 1 mg/mouse of BrdU, starting on day 9 post immuniozation (p.i.), for the following 9 days, and were sacrificed on day 18. Increased numbers of newly generated BrdU+Olig-2+CC1+ mature oligodendrocytes were observed in the lumbar spinal cords of anti-BMP-2/4 mAb-treated R-EAE mice (b) compared to those in IC-treated R-EAE mice (a) on day 18 p.i. Scale bar: 50 µm; **P < 0.01.

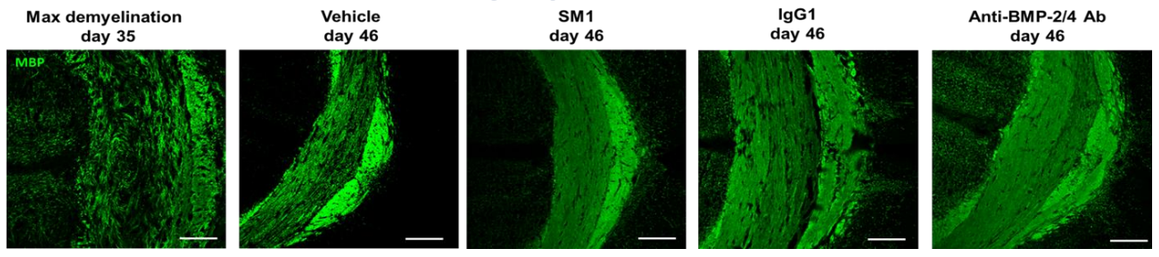
BMP-2/4 blockade induces remyelination in the spinal cord of R-EAE

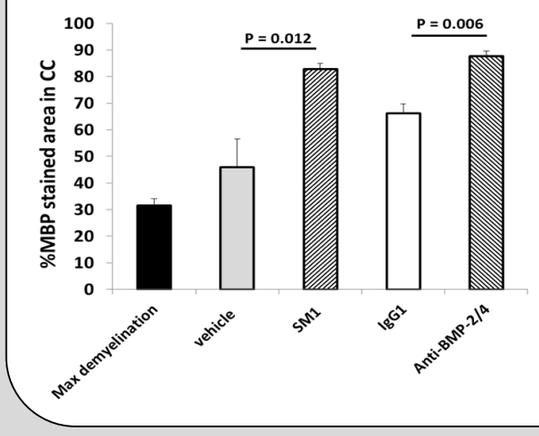




Treatment of R-EAE- mice with anti-BMP-2/4 Ab increased the number of remyelinated axons (A2, 0.8 < g-ratio < 1) and reduced the number of fully demyelinated axons (A3, g-ratio = 1). The number of vesiculated axons during the process of demyelination, was also significantly reduced in the anti- BMP-2/4 mAb (A4). * P < 0.05, ** P < 0.01, chi-squared test, N = 5 mice/group, >200 axons per animal. Scale bar = 5 µm.

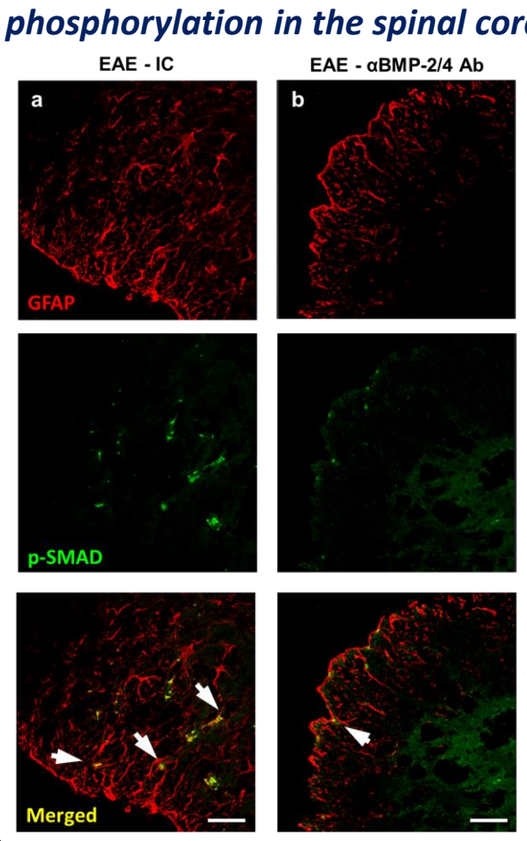
Anti- BMP-2/4 mAb and SM1 promote remyelination in the corpus callosum of cuprizone- induced mice



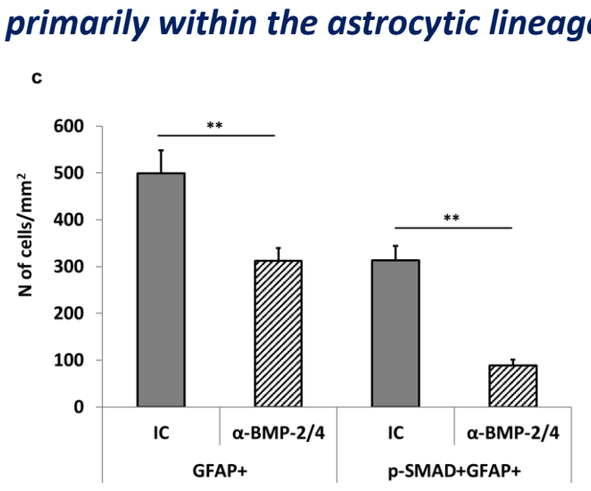


C57BL/6 mice were fed 0.2% cuprizone for 5 weeks (day 35, maximum demyelination). The anti- BMP-2/4 neutralizing mAb was IV administrated (30 µg/mouse) on day 15 after the initiation of the cuprizone diet. A control group was IV injected with 30 µg/mouse of mouse IgG1. SM1 (500 µg/mouse) or vehicle alone (5% DMSO in PBS) was daily IP administrated from day 15 for the 30 following days. Mice were sacrificed 10 days after resuming the regular diet (recovery phase, day 46), N = 5 in each group. (scale bar = 100µm).

Blockade of BMP-2/4 signaling in R-EAE reduces SMAD1/5/8 phosphorylation in the spinal cord, primarily within the astrocytic lineage



Scale bar 50µm



Co-localization of GFAP and p-SMAD1/5/8 staining demonstrates that most of the p-SMAD$^+$ cells are of GFAP$^+$ origin. Quantification demonstrates reduced numbers of both total GFAP$^+$ and double-stained p-SMAD$^+$GFAP$^+$ in the anti-BMP-2/4 Ab- treated group (b), compared to the IC- treated group (a), N = 3 mice/ group, scale bar = 50 µm, **P < 0.01.

CONCLUSION

Systemic blockage of BMP-2/4 signaling induces remyelination in both the inflammatory R-EAE model and the toxic- cuprizone model, thus leads it to be a therapeutic target for remyelination enhancement in MS.