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J Neuroimmunol. Author manuscript; available in PMC 2016 August 17.

Published in final edited form as:

J Neuroimmunol. 2011 January ; 230(1-2): 164–168. doi:10.1016/j.jneuroim.2010.10.028.

TGF-β signaling is altered in the peripheral blood of subjects with multiple sclerosis

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Abstract

Multiple sclerosis (MS) is a central nervous system inflammatory disorder with evidence of peripheral immune dysregulation. Abnormalities of the immune suppressive cytokine TGF-β have been reported, but not fully defined, in MS. Through a pathway-focused expression profiling of the peripheral blood, we found abnormalities of TGF-βRII, SMAD4 and SMAD7 expression in subjects with MS, and reduction in the levels of TGF-β regulated genes, indicating an overall reduction in TGF-β signaling in MS. The response to exogenous TGF-β was intact, however, indicating an extrinsic defect of TGF-β signaling in MS. These results indicate that TGF-β control is diminished in MS.

Keywords

multiple sclerosis; inflammation; TGF-β; SMAD7

1. Introduction

Multiple sclerosis (MS) is a chronic disabling inflammatory disease of the central nervous system, where the presence of activated autoreactive T cells in the periphery suggests that defects in immune regulation may contribute to the pathogenesis (1). Transforming growth factor-β (TGF-β) is a pleiotropic cytokine with immune regulatory properties (2). TGF-β plays a key role in suppressing autoreactive T cell responses, either as a soluble mediator of immune suppression or as a membrane-bound effector molecule of $CD4^+CD25^+$ regulatory T cells (3). Abnormalities of TGF-β production have been reported in MS, but the results are conflicting and as yet unresolved (4–7). More recently, gene expression profiling studies of MS have identified altered expression of the TGF-β-regulated gene SMAD7 (8, 9), suggesting that abnormalities of TGF-β signaling are detectable at the transcriptional level and may be informative in understanding the role of TGF-β in MS.

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The integrity of the TGF-β signaling pathway in MS has yet to be fully characterized. TGF-β exerts its immune suppressive properties through the SMAD signaling cascade (10). Upon binding of TGF-β to its receptor, composed of TGF-βRI and TGF-βRII subunits, receptor-regulated SMAD2 and SMAD3 are recruited and phosphorylated. The SMAD2/3 heterodimer then complexes with the common-mediator SMAD4, leading to nuclear translocation where the SMAD complex controls the transcriptional activity of a number of down-stream target genes including the inhibitory SMAD7, platelet activating inhibitor, and cyclin dependent kinase inhibitor p21 (11).

To assess the integrity of the TGF-β signaling pathway in MS, we compared peripheral blood mononuclear cells (PBMC) from subjects with MS and healthy donors by TGF-β pathway-focused expression profiling, and by assessing TGF-β signaling capacity.

2. Materials and Methods

Samples

Blood samples were collected from 25 subjects with untreated MS and 23 healthy donors. Subjects with MS fulfilled established diagnostic criteria (12). Demographics and magnetic resonance imaging data for subjects with MS are given in the Table. Subjects with MS and healthy donors were matched for age (37 +/− 7 vs. 37 +/− 13 years), gender (male to female ratio: 0.92 vs. 0.88), and included two African American subjects in each group. All subjects provided written, informed consent as part of a research protocol reviewed and approved by an NIH Clinical Center Institutional Review Board. PBMC were isolated by centrifugation of blood samples over Ficoll-Hypaque gradients (Lonza Walkersville, Walkersville, MD). The cells were viably cryopreserved prior to testing. Purified $CD4^+$ T cells were isolated from PBMC using the Human $CD4^+$ T cell Isolation Kit II (Miltenyi, Auburn, CA) according to manufacturer's guidelines. Purity of negatively selected $CD4^+$ cells was verified by flow cytometry and was consistently above 93%.

Real-time RT-PCR

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to manufacturer's guidelines and cDNA was synthesized by reverse transcription using TaqMan RT-PCR Kit reagents and random hexamer primers from Applied Biosystems (Foster City, CA). Relative quantification of mRNA was performed using the comparative threshold cycle method with HPRT as endogenous control. Primer and probe sets for SMAD3, SMAD4, SMAD7, TGF-BRII, p21 and HPRT were obtained from Applied Biosystems. To determine relative expression levels, the following formula was used: target gene expression = $2^{-(Ct[target]-Ct[HPRT])}$.

Immunoblot

PBMC were lysed in Tween 20 lysis buffer: 25mM Tris/Hepes pH 8.0, 10mM NaCl, 2mM EDTA, 1mM PMSF, 0.5% Tween 20 and a cocktail of protease inhibitors (Sigma-Aldrich, St. Louis, MO). After 15 minutes on ice, the mixture was centrifuged and the supernatant was collected. Protein concentration was measured using Quickstart Bradford Assay (Biorad, Hercules, CA). From each protein sample, 50μg was electrophoresed through a

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NuPAGE® 12% Bis-Tris gel (Invitrogen, Carlsbad, CA), transferred to a PVDF membrane (Invitrogen), then dried and blocked with 3% BSA in TBS. The membrane was probed with anti-SMAD7 antibody (Imgenex, San Diego, CA) followed by horseradish peroxidase-conjugated rabbit IgG (Cell Signaling, Danvers, MA). The membrane was visualized by chemiluminescence (SuperSignal West Pico Chemiluminescent substrate, Thermo Scientific, Rockford, IL). SMAD7 protein levels were quantified by densitometry (1D image analysis software, Kodak, Rochester, NY) normalized to β-actin.

TGF-β stimulation assay

PBMC from healthy donors or subjects with MS were plated in 24-well plates containing serum-free media for 8 hrs. Cells were then treated with recombinant human TGF-β1 (Peprotech, Rocky Hill, NJ) at concentrations of 0.1, 1 and 10 ng/ml or were left untreated. Cells were harvested and RNA isolated after 1.5 hrs of TGF-β1 treatment.

Statistical analyses

Kolmogorov-Smirnov test was used as a test of normality. Mann-Whitney test was used for between-group comparisons. Wilcoxon signed rank test was used for before-after comparisons. Pearson correlation was used to test the correlation between SMAD7 and p21 expression. Statistical analyses were performed using Prism software (GraphPad Software, San Diego, CA).

3. Results

TGF-β signaling pathway is altered in MS

The transcriptional activities of several key components of the TGF-β signaling pathway, TGF-βRII, SMAD3, SMAD4 and SMAD7, were measured by real-time RT-PCR. The TGF-β signaling pathway was significantly altered at multiple levels in PBMC from subjects with MS compared to healthy donors (Figure 1A). Subjects with MS showed higher levels of the TGF-β receptor subunit TGF-βRII and higher levels of the common-mediator SMAD4 compared to healthy donors ($p = 0.0017$ and $p < 0.0001$, respectively). The receptor-regulated SMAD3 expression was unchanged. By contrast, levels of the inhibitory SMAD7, a TGF-β regulated gene, was significantly lower in PBMC from subjects with MS ($p < 0.0001$). Decreased SMAD7 protein expression in PBMC from subjects with MS was confirmed by immunoblot (Figure 1B). Purified CD4+ T cells from subjects with MS also showed significantly lower SMAD7 expression compared to healthy donors (Figure 1C). Taken together, these results indicated a perturbation of TGF-β signaling in subjects with MS.

TGF-β control of transcriptional activity is impaired in MS

The transcriptional activity of the cyclin dependent kinase inhibitor p21, a TGF-β-regulated gene, was examined to gauge the net impact of these changes in MS with respect to TGF-β control of down-stream transcriptional events. The transcriptional activity of the cyclin dependent kinase inhibitor p21 was significantly lower in subjects with MS compared to healthy donors ($p < 0.0001$, Figure 2A). There was a strong correlation between the expression levels of p21 and SMAD7 in PBMC from subjects with MS ($p < 0.0001$, $R^2 =$

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0.65, Figure 2B). The parallel reduction in the transcriptional activities of SMAD7 and the cyclin dependent kinase inhibitor p21, both TGF-β-regulated genes, indicated that the overall perturbation of TGF-β in MS was that of reduced TGF-β signaling.

Response to ex vivo TGF-β is intact in MS

To determine whether the observed changes represent an intrinsic defect of TGF-β signaling, PBMC from subjects with MS were tested by an *ex vivo* TGF-β stimulation assay using SMAD7 transcriptional activity as a marker of TGF-β signaling. As shown in Figure 3A, healthy donor PBMC demonstrated dose-dependent induction of SMAD7 expression in response to recombinant human TGF-β1. Likewise, PBMC from subjects with MS showed comparable dose-dependent induction of SMAD7 to TGF-β1 stimulation. Thus e*x vivo* TGF-β stimulation assay showed that the TGF-β signaling capacity is essentially intact in PBMC from subjects with MS. Interestingly, overnight incubation in serum-free media to deprive cells of TGF-β exposure resulted in a drop in SMAD7 expression in healthy donor PBMC down to levels comparable to that of MS (Figure 3B). These results, taken together, suggest that the changes in the TGF-β signaling pathway observed in MS are more likely explained by an extrinsic (i.e. decreased TGF-β exposure *in vivo*) rather than an intrinsic defect of TGF-β signaling.

Reduced SMAD7 expression in MS is not altered by disease modifying therapies

Finally, we asked whether disease-modifying treatments in MS affect TGF-β signaling in MS. As shown in Figure 4, SMAD7 expression in MS remained low after 6 months of disease modifying therapy, indicating that neither interferon beta nor glatiramer acetate significantly impact TGF-β signaling in MS. SMAD7 expression remained unchanged in healthy donor PBMC over a similar time period, suggesting that SMAD7 expression is a relatively stable marker over time in healthy donor PBMC.

4. Discussion

The results of this study indicate that TGF-β signaling is altered in MS, as evidenced by changes in the transcriptional activity of multiple elements of the TGF-β signaling pathway. Low levels of SMAD7 and p21 expression reflect reduced TGF-β transcriptional control and increased TGF-βRII and SMAD4 likely represent compensatory up-regulation. These changes in the TGF-β signaling are most likely explained by reduced TGF-β signaling in MS. The response to exogenous TGF-β1 was intact in PBMC from subjects with MS, however, arguing against an intrinsic defect or inhibition of TGF-β signal transduction. Possible explanations for reduced TGF-β signaling despite an intact TGF-β signaling capacity in MS include decreased *in vivo* exposure to TGF-β or the presence of a serum inhibitor of TGF-β. Previous studies have shown decreased TGF-β production in MS. Mokhtarian et al., reported decreased TGF-β production in cultured PBMC from subjects with MS compared to healthy donors (4). Correale et al., reported that T cell clones isolated from subjects with MS during exacerbation were less likely to produce TGF-β upon proteolipid protein stimulation than those obtained during remission (5). However, other studies have contradicted these findings by reporting increased TGF-β production in MS (6, 7). The results of our study support decreased TGF-β input in MS based on a pathway-

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focused expression profiling that was consistent with reduced TGF-β signaling. The results of our study also confirm findings of reduced SMAD7 levels in MS reported in two independent gene expression profiling studies (8, 9).

These results have several important implications with respect to the pathogenesis of MS. A decrease in TGF-β signaling would be predicted to have a negative impact on immune regulation. In animal models, the loss of TGF-β signaling in T cells, either by the loss of TGF-β1 or by the loss of functional TGF-βRII, leads to multi-organ inflammatory disease (13, 14). The suppressive function of the immune suppressive $CD4^+CD25^+$ regulatory T cells are particularly affected, as $CD4^+CD25^+$ regulatory T cell-mediated control of inflammation is impaired in the setting of defective TGF-β signaling (15). In humans, defective TGF-β signaling has been implicated in the pathogenesis of inflammatory bowel disease (16). In addition, we have recently shown that aberrant TGF-β signaling is a feature of HTLV-1-assosicated myelopathy/tropical spastic paraparesis, a chronic central nervous system inflammatory disorder (17). Impaired TGF-β signaling in MS would therefore be predicted to contribute to central nervous system inflammation. In this regard, it is noteworthy that impaired $CD4^+CD25^+$ regulatory T cell function has been reported in MS (18). The underlying cause of regulatory T cell dysfunction in MS has yet to be clarified, and it may be informative to examine TGF-β expression/production by $CD4^+CD25^+$ regulatory T cells in MS.

Abnormalities of TGF-β in MS may represent a potential target for therapy(19). Our results suggest that the current first-line disease modifying therapies in MS do not impact TGF-β abnormalities in MS. Neither interferon beta nor glatiramer acetate significantly normalized SMAD7 expression in MS after 6 months of therapy. Pharmacologic treatment to augment TGF-β presents unique challenges, as was shown in a previous phase I trial of TGF-β2 administration in subjects with progressive MS where reversible nephrotoxicity was likely a mechanism-related adverse event (19). Transgenic animal models over-expressing TGF-β exhibit propensity for pathologic fibrosis, underscoring the role of TGF-β as a pleiotropic cytokine that also plays a key role in extracellular matrix formation(20). Further work is needed to better understand the source of TGF-β critical for regulation of T cells in MS, whether that may be $CD4^+CD25^+$ regulatory T cells or another regulatory cell subset. Recently, Kleiter et al., reported increased SMAD7 expression in subjects with MS during acute clinical relapse (21). We found no correlation between SMAD7 expression and acute CNS inflammatory activity as measured by gadolinium contrast-enhanced MRI (Table), and thus our results appear to contradict the findings of increased SMAD7 in MS. It remains possible that these contradictory results reflect the heterogeneity of MS.

In summary, PBMC from subjects with MS exhibit abnormalities at multiple levels of the TGF-β signaling pathway. TGF-β control of down-stream transcriptional activity is impaired in MS. Given the demonstration of an essentially intact TGF-β signaling capacity in response to exogenous TGF-β, a likely explanation for the observed changes in the TGF-β signaling pathway in MS is decreased *in vivo* exposure to TGF-β. The known role of TGF-β in the control of inflammation suggests that impaired TGF-β control of T cells may contribute to the loss of immune tolerance in MS.

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Acknowledgments

We thank the Drs. Frank Ruscetti, Daniel Bertolette, and Karen Yao for helpful advice. This work was supported by the Intramural Research Program of the NIH, NINDS.

References

1. Zhang J, Markovic-Plese S, Lacet B, Raus J, Weiner HL, Hafler DA. Increased frequency of interleukin 2-responsive T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. J Exp Med. 1994; 179:973–984. [PubMed: 7509366]
2. Gorelik L, Flavell RA. Transforming growth factor-beta in T-cell biology. Nat Rev Immunol. 2002; 2:46–53. [PubMed: 11905837]
3. Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med. 2001; 194:629–644. [PubMed: 11535631]
4. Mokhtarian F, Shi Y, Shirazian D, Morgante L, Miller A, Grob D. Defective production of anti-inflammatory cytokine, TGF-beta by T cell lines of patients with active multiple sclerosis. J Immunol. 1994; 152:6003–6010. [PubMed: 8207225]
5. Correale J, Gilmore W, McMillan M, Li S, McCarthy K, Le T, Weiner LP. Patterns of cytokine secretion by autoreactive proteolipid protein-specific T cell clones during the course of multiple sclerosis. J Immunol. 1995; 154:2959–2968. [PubMed: 7533188]
6. Monteyne P, Guillaume B, Sindic CJ. B7-1 (CD80), B7-2 (CD86), interleukin-12 and transforming growth factor-beta mRNA expression in CSF and peripheral blood mononuclear cells from multiple sclerosis patients. J Neuroimmunol. 1998; 91:198–203. [PubMed: 9846836]
7. Link J, Soderstrom M, Olsson T, Hojeberg B, Ljungdahl A, Link H. Increased transforming growth factor-beta, interleukin-4, and interferon-gamma in multiple sclerosis. Ann Neurol. 1994; 36:379–386. [PubMed: 8080246]
8. Achiron A, Gurevich M, Friedman N, Kaminski N, Mandel M. Blood transcriptional signatures of multiple sclerosis: unique gene expression of disease activity. Ann Neurol. 2004; 55:410–417. [PubMed: 14991819]
9. Bomprezzi R, Ringner M, Kim S, Bittner ML, Khan J, Chen Y, Elkahloun A, Yu A, Bielekova B, Meltzer PS, Martin R, McFarland HF, Trent JM. Gene expression profile in multiple sclerosis patients and healthy controls: identifying pathways relevant to disease. Human molecular genetics. 2003; 12:2191–2199. [PubMed: 12915464]
10. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell. 2003; 113:685–700. [PubMed: 12809600]
11. Datto MB, Li Y, Panus JF, Howe DJ, Xiong Y, Wang XF. Transforming growth factor beta induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. Proc Natl Acad Sci U S A. 1995; 92:5545–5549. [PubMed: 7777546]
12. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van den Noort S, Weinshenker BY, Wolinsky JS. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol. 2001; 50:121–127. [PubMed: 11456302]
13. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature. 1992; 359:693–699. [PubMed: 1436033]
14. Gorelik L, Flavell RA. Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. Immunity. 2000; 12:171–181. [PubMed: 10714683]
15. Fahlen L, Read S, Gorelik L, Hurst SD, Coffman RL, Flavell RA, Powrie F. T cells that cannot respond to TGF-beta escape control by CD4(+)CD25(+) regulatory T cells. J Exp Med. 2005; 201:737–746. [PubMed: 15753207]

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16. Monteleone G, Kumberova A, Croft NM, McKenzie C, Steer HW, MacDonald TT. Blocking Smad7 restores TGF-beta1 signaling in chronic inflammatory bowel disease. J Clin Invest. 2001; 108:601–609. [PubMed: 11518734]

17. Grant C, Oh U, Yao K, Yamano Y, Jacobson S. Dysregulation of TGF-beta signaling and regulatory and effector T-cell function in virus-induced neuroinflammatory disease. Blood. 2008; 111:5601–5609. [PubMed: 18326816]

18. Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. J Exp Med. 2004; 199:971–979. [PubMed: 15067033]

19. Calabresi PA, Fields NS, Maloni HW, Hanham A, Carlino J, Moore J, Levin MC, Dhib-Jalbut S, Tranquill LR, Austin H, McFarland HF, Racke MK. Phase 1 trial of transforming growth factor beta 2 in chronic progressive MS. Neurology. 1998; 51:289–292. [PubMed: 9674825]

20. Sanderson N, Factor V, Nagy P, Kopp J, Kondaiah P, Wakefield L, Roberts AB, Sporn MB, Thorgeirsson SS. Hepatic expression of mature transforming growth factor beta 1 in transgenic mice results in multiple tissue lesions. Proc Natl Acad Sci U S A. 1995; 92:2572–2576. [PubMed: 7708687]

21. Kleiter I, Song J, Lukas D, Hasan M, Neumann B, Croxford AL, Pedre X, Hovelmeyer N, Yogev N, Mildner A, Prinz M, Wiese E, Reifenberg K, Bittner S, Wiendl H, Steinman L, Becker C, Bogdahn U, Neurath MF, Steinbrecher A, Waisman A. Smad7 in T cells drives T helper 1 responses in multiple sclerosis and experimental autoimmune encephalomyelitis. Brain. 133:1067–1081. [PubMed: 20354004]

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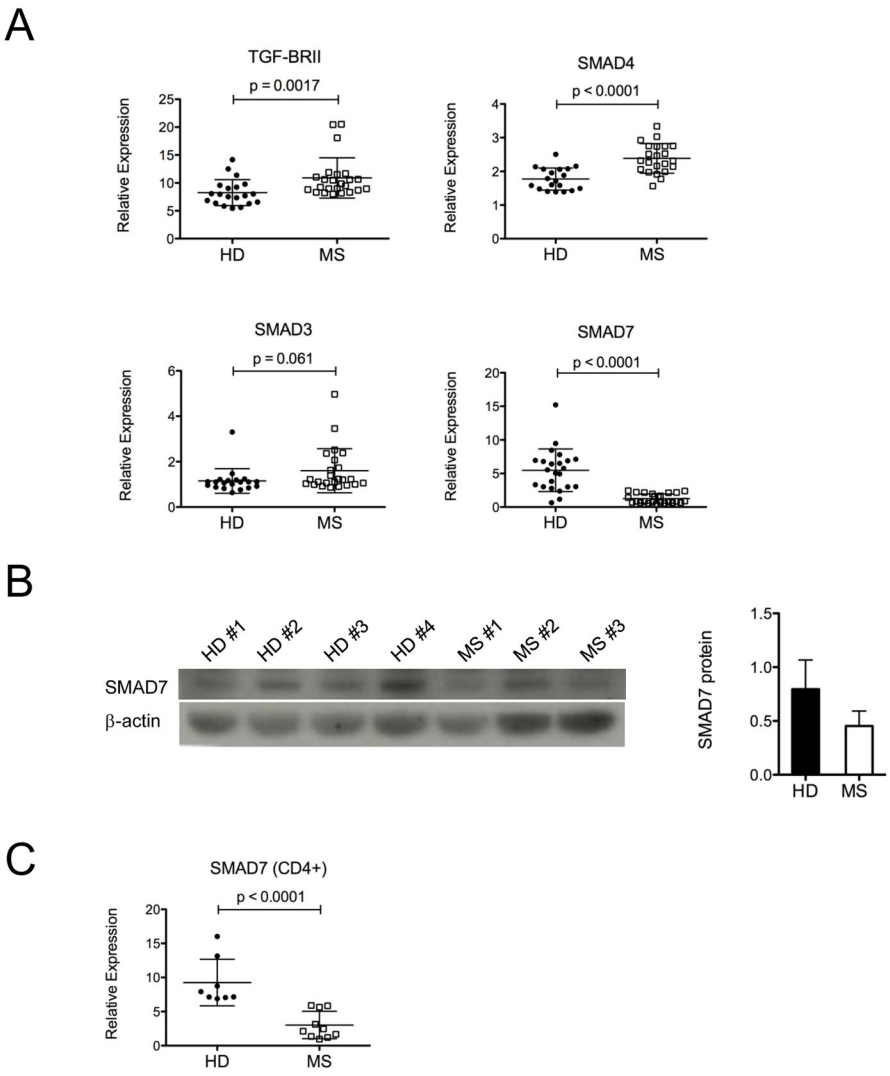

Figure 1. TGF-β signaling pathway is altered in MS

A) Transcriptional activity (Relative Expression) of TGF-βRII, SMAD4, SMAD3 and SMAD7 in PBMC of healthy donors (HD) and subjects with multiple sclerosis (MS). B) SMAD7 protein immunoblot (left) for healthy donors (HD#1–4) and subjects with MS (MS#1–3), and quantitation by densitometry (mean +/− standard deviation, right). C) SMAD7 transcriptional activity (Relative Expression) in purified CD4+ T cells from healthy donors (HD) and subjects with MS.

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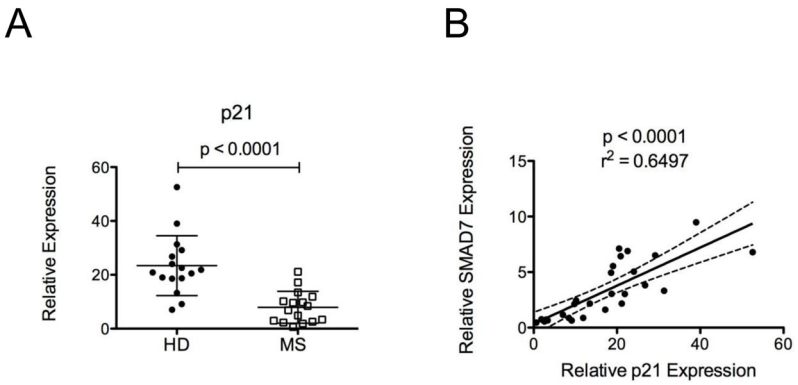

Figure 2. TGF-β control of transcriptional activity is impaired in MS

A) Cyclin dependent kinase inhibitor p21 transcriptional activity (Relative Expression) in PBMC from healthy donors (HD) and subjects with multiple sclerosis (MS). B) Correlation between p21 and SMAD7 transcriptional activity in subjects with MS.

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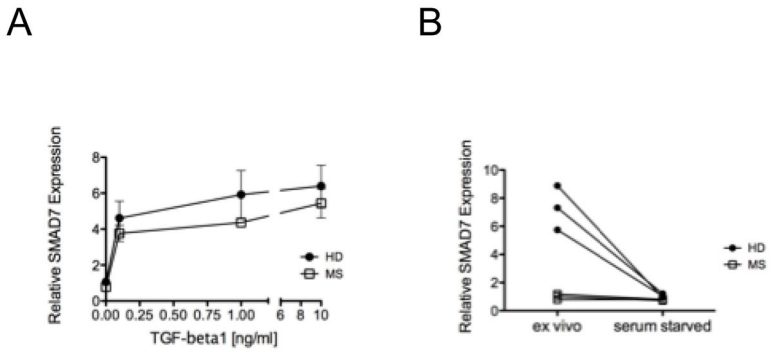

Figure 3. Response to ex vivo TGF-β is intact in MS

A) SMAD7 levels (Relative SMAD7 Expression) as a function of increasing doses of recombinant human TGF-b1 in PBMC from healthy donors (n = 3; solid circles) and MS (n = 3; open squares). B) SMAD7 levels directly ex vivo and after overnight serum-deprivation (serum starved) in PBMC from healthy donors (n = 3; solid circles) and subjects with MS (n = 3; open squares).

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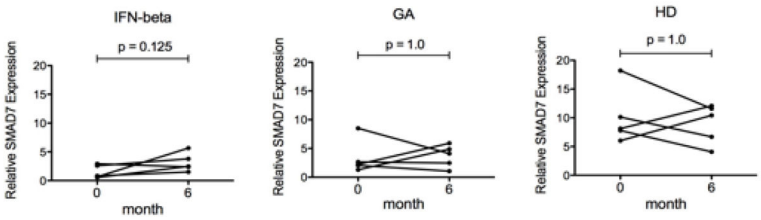

Figure 4. Reduced SMAD7 expression in MS is not altered by disease modifying therapies

SMAD7 levels in subjects with multiple sclerosis before (month 0) and after six months (month 6) of interferon-beta (IFN-beta) therapy (left), and before and after glatiramer acetate (GA) therapy (center). Longitudinal (6 months) SMAD7 levels in healthy donor PBMC (right).

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Table 1

MS subject demographics with corresponding magnetic resonance imaging and SMAD7 data.

Subject	Sex	Age	Gd+ lesions on MRI	SMAD7
MS1	M	47		1.67
MS2	M	33	+	0.63
MS3	M	33	+	0.75
MS4	F	41	+	2.16
MS5	F	36	+	0.87
MS6	F	41		0.73
MS7	M	28	+	1.62
MS8	M	25	+	2.13
MS9	F	24	+	0.66
MS10	M	44		0.90
MS11	M	33	+	0.82
MS12	F	33		0.71
MS13	F	31	+	0.56
MS14	M	40	+	0.65
MS15	F	40	+	0.88
MS16	F	38	+	2.17
MS17	F	33	+	0.45
MS18	M	52	+	2.45
MS19	F	35		0.69
MS20	M	49	+	0.55
MS21	M	46		1.95
MS22	M	42		2.39
MS23	F	42	+	1.47
MS24	F	31	+	1.42
MS25	F	44	+	2.21

M = male. F = female. Gd+ lesions on MRI = “+” indicates the presence of two or more gadolinium contrast-enhancing lesions on magnetic resonance imaging at the time of peripheral blood sampling. SMAD7 = relative SMAD7 mRNA expression in PBMC.