ANNALS of Clinical and Translational Neurology
Open Access
ANA American Neurological Association since 1875

BRIEF COMMUNICATION

CD4+ T cells from multiple sclerosis patients respond to a commensal-derived antigen

Joseph N. Burgess, Anudeep B. Pant, Lloyd H. Kasper & Sara Colpitts Brass

Department of Microbiology and Immunology, Geisel School of Medicine, Dartmouth College, Hanover 03755, New Hampshire

Correspondence
Sara Colpitts Brass, Dartmouth College, 66 College St., 706 Remsen Bldg. Hanover, NH 03755. Tel: 603 650 1067; Fax: 603 650 1728; E-mail: Sara.Colpitts.Brass@Dartmouth.edu

Funding Information
This work was supported by a grant from the NIH (AI110170 to LHK).

Received: 19 April 2017; Revised: 31 July 2017; Accepted: 17 August 2017

Annals of Clinical and Translational Neurology **2017; 4(11): 825–829**

doi: 10.1002/acn3.465

Abstract

Multiple sclerosis, an immune-mediated disease of the central nervous system, is characterized by the impaired function of regulatory cells that fail to suppress self-reactive effector cells. We have previously found that polysaccharide A, a capsular antigen derived from the human gut commensal *Bacteroides fragilis*, can induce a population of regulatory T cells. Herein, we demonstrate that naïve T cells isolated from patients with multiple sclerosis have the capacity to acquire regulatory characteristics when stimulated *in vitro* with polysaccharide A. This study demonstrates the amplification of a regulatory T cell response by a gut-derived commensal antigen in those with multiple sclerosis.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) associated with demyelination, neuronal transection, and progressive disability. The most common presentation of MS is relapsing-remitting MS that is associated with periods of active inflammation and demyelination that can last for days or months followed by periods of remission and remyelination with decreased disease severity. It is believed that the fluctuation between relapse and remission is greatly influenced by the balance of self-reactive effector T cells and regulatory T cells (T_{regs}). Importantly, studies have shown that while MS patients harbor normal frequencies of T_{regs}, their production of IL-10 and overall suppressive capacity is significantly reduced allowing for increased inflammation driven by effector cells.[1–3] For many years, the immunopathogenesis of this condition was attributed to an imbalance of Th1/Th2 polarization in which the enhanced Th1 response was associated with the production of IFNγ and TNFα. More recent studies in both humans and mice have alternatively indicated that IL-23 and IL-17 are the principal proinflammatory cytokines responsible for disease progression.

While the etiology of MS is poorly understood, recent studies have established a connection between inflammation in the CNS and the microbial composition of the gut (referred to as the gut microbiome).[4] Importantly, MS patients with active disease and those who have yet to undergo treatment have altered abundances of specific microflora within their gut microbiome, whereas patients in remission or undergoing treatment, respectively, harbor microflora that is more similar to controls.[5,6] Thus, a reduction in disease severity in the brain positively correlated with the correction of altered gut microflora (dysbiosis). The ability of gut microbes to modulate neuroimmune activity has been well documented. In the mouse model of MS (experimental autoimmune encephalomyelitis; EAE), segmented filamentous bacteria promote the development of disease, while *Bacteroides fragilis* has anti-inflammatory properties.[7–9] In humans, *Clostridium perfringens* has been associated with the onset of neuromyelitis optica spectrum disorder, an MS-like disease.[10]

B. fragilis is an anaerobic commensal that comprises 0.5–1.0% of the human colonic bacterial microflora in all mankind.[11] *B. fragilis* produces eight different polysaccharides of which polysaccharide A (PSA) has potent immunomodulatory properties. Orally administered PSA protects against EAE and experimental colitis by

eliciting T_{regs} with IL-10-dependent immunosuppressive function.[12,13] While it had previously been demonstrated that PSA acts via TLR2 to drive the induction of T_{regs} in mice,[14] we have recently shown that PSA can induce the differentiation of T_{regs} when added to *in vitro* co-cultures of dendritic cells (DCs) and naïve T cells isolated from the blood of healthy donors.[15] In this study, we determined if PSA could similarly drive the differentiation of T_{regs} from naïve T cells isolated from patients with MS.

Methods

Fresh blood was collected from healthy volunteers ($n = 18$) (Dartmouth Hitchcock Memorial Hospital; Lebanon, NH) and patients with MS ($n = 18$) (Concord Hospital; Concord, NH). Patient demographics are detailed in Table 1. The protocol for the study was approved by the Institutional Review Boards at both locations, and informed consent was obtained from each subject. Due to the geographical distance between Concord Hospital and Dartmouth College, blood was stored overnight at room temperature under gentle agitation. Healthy control blood was treated identically despite its local acquisition. Peripheral blood mononuclear cells were isolated using a ficoll concentration gradient (ThermoFisher). Naïve T cells ($CD4^+CD45RA^+$) and DCs ($CD11c^+$) were sorted using magnetic-associated cell sorting per the manufacturer's instructions (Miltenyi). Forty thousand T cells were co-cultured with 10,000 DCs and stimulated with or without PSA (25 μg/mL) in AIM V media supplemented with 5% human serum and recombinant IL-2 (100 U/mL) at 37°C and 5% CO_2. After 5 days, supernatants were harvested for detection of IL-10 by ELISA (Biolegend), and the cells were assayed for Foxp3 expression by flow cytometry (eBioscience) and analyzed using FlowJo (TreeStar). Statistical significance was determined using Prism software (GraphPad).

Results

We used both phenotypic and functional parameters to examine the conversion of naïve T cells to T_{regs} following *in vitro* co-culture with DCs and PSA. When T cells isolated from healthy donors were cultured in the presence of PSA, we found a significant increase in the frequency of $Foxp3^+$ T cells as previously shown ($P = 0.0304$) (Fig. 1A).[15] T cells were also isolated from MS patients that were either naive to treatment (MS; $n = 10$) or actively treated with glatiramer acetate (Copaxone®) (MS-GA; $n = 8$). We found a significant increase in the frequency of $Foxp3^+$ T cells when cells isolated from MS patients were stimulated with PSA

Table 1. Donor demographics. No significant differences in the age of controls versus MS patients were identified using one-way ANOVA for statistical analysis. S.D; standard deviation

		MS patients	
N (Sex)	Healthy controls 18(F)	Untreated 10(F)	GA 5(F)/3(M)
Age Range	25-54	29-67	30-63
Mean age ± S.D.	42.3 ± 10.15	49.1 ± 11.7	50.9 ± 11.38

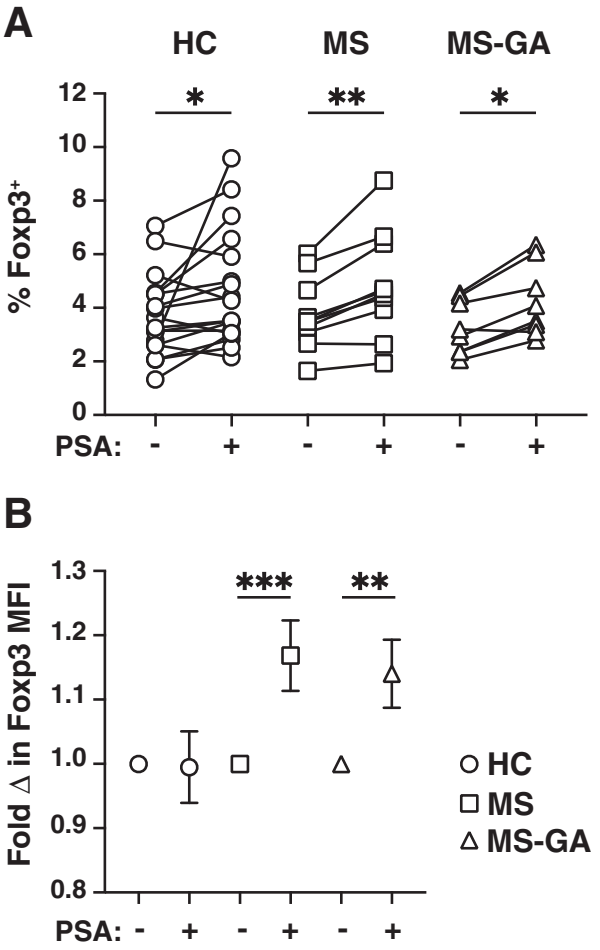

Figure 1. Foxp3 expression in T cells stimulated with PSA. Naïve T cells isolated from the indicated donors (healthy controls [HC/○, untreated MS patients [MS/ □], and GA-treated MS patients [MS-GA/ △]) were cultured *in vitro* for 5 days in the presence or absence of PSA. Foxp3 expression was determined by intracellular staining. (A) Data are presented as the % $Foxp3^+$ of the total live $CD4^+$ population. Statistical analysis was determined using a paired *t* test (nonparametric Wilcoxon matched-pairs signed-rank test). * $P < 0.05$; ** $P < 0.01$ (B) The fold change in the MFI of Foxp3 was calculated by dividing the average MFI of Foxp3 in the PSA-treated wells by the average MFI of Foxp3 in untreated wells. Statistical analysis was determined using an unpaired *t* test (nonparametric Mann–Whitney test). ** $P < 0.01$; ***$P < 0.001$.

($P = 0.0039$). Similarly, PSA could induce T_{reg} conversion in naive T cells isolated from patients undergoing treatment with GA ($P = 0.0156$). As an additional parameter to ascertain T_{reg} induction, we measured the mean fluorescence intensity (MFI) of Foxp3 in the $Foxp3^+$ T_{reg} population induced by PSA. Since samples were collected on different days using different instrument settings, we calculated the fold change in the intensity of Foxp3 relative to the cells cultured without PSA. We found a significant increase in the fold change in the MFI of Foxp3 in both cohorts of MS patients ($P = 0.0006$ [untreated] and $P = 0.0057$ [GA-treated]) (Fig. 1B). Despite the small sample size of our study, these data suggest that a single gut-derived microbial antigen has the capacity to convert naive T cells isolated from patients with MS to a T_{reg} phenotype.

This result is of potentially important clinical value since T_{reg} function is known to be impaired in patients with MS.[1–3] Since suppressive function often corresponds to the production of IL-10, we next determined if stimulation with PSA resulted in an increase in IL-10 production. Similar to our previous studies, there was a significant increase in IL-10 production when cells from healthy donors were stimulated *in vitro* with PSA ($P = 0.0034$) (Fig. 2). Importantly, cells from untreated MS patients also produced significantly more IL-10 in the presence of PSA ($P = 0.0039$). T cells isolated from some of the GA-treated patients demonstrated an increase in the production of IL-10. While these findings did not reach statistical significance, there was an overall trend toward increased IL-10 in cells treated with PSA compared to control ($P = 0.0781$). Together, these findings suggest that naïve T cells from MS patients, particularly those naive to treatment, have the capacity to acquire immunosuppressive function in response to this isolated commensal antigen that could potentially correct the established defects in T_{reg} function during MS. Furthermore, the observed trend would suggest that upregulation of IL-10 by PSA may amplify T_{reg} conversion in combination with established MS therapeutics.

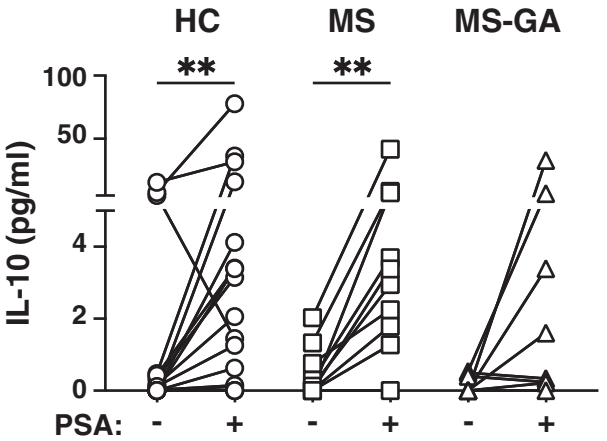

Figure 2. IL-10 production by T cells stimulated with PSA. Naïve T cells isolated from the indicated donors were cultured *in vitro* for 5 days in the presence or absence of PSA. IL-10 (pg/mL) was measured in the supernatants by ELISA. Statistical analysis was determined using a paired *t* test (nonparametric Wilcoxon matched-pairs signed-rank test). ** $P < 0.01$.

Discussion

The composition of the mammalian gut microbiome has been shown to have various effects on immune function and other bodily processes. Intestinal dysbiosis has been identified in multiple autoimmune disorders affecting not only the CNS but a wide range of organs. Specifically relating to the CNS, dysbiosis appears in both EAE and human MS. Recent studies show that patients exhibit a shift in the relative abundances of certain genera.[4–6] For example, those with MS harbor increased levels of *Blautia* (from the phylum *Firmicutes*) and decreased *Parabacteroides* (from the phylum *Bacteroidetes*) compared to healthy controls.[5] Such shifts could influence the overall ratio of *Firmicutes* to *Bacteroidetes* which is known to have downstream physiological and immunological effects. Together these data indicate that the gut microbiome is an important and critical organ that may be directly involved in the pathogenesis of CNS demyelinating disease. Other CNS conditions including autism, Parksinson's disease, depression, and both stroke and spinal cord injury are associated with gut dysbiosis and, in the case of spinal cord injury, can demonstrate improvement in motor function and repair of the nervous system when addressed.[16–20] The association between immune function, nervous system repair, and the mammalian microbiome may suggest that novel therapies for inflammatory diseases of the CNS, such as MS, could be found in the human gut microbiome.

The human gut commensal *B. fragilis* has been shown to have a beneficial effect in the mouse model of MS that is dependent on the expression of PSA.[9] Similarly, purified PSA can significantly reduce disease severity when orally administered either prophylactically or therapeutically using IL-10-dependent mechanisms.[12] *In vitro* studies using PBMCs from healthy human donors have shown that PSA stimulates DCs to induce immunosuppressive $Foxp3^+$ $T_{regs.}$[15] Unlike murine studies,[21] we had previously found that plasmacytoid DCs in combination with PSA were unable to induce Foxp3 expression in human $CD4^+$ T cells.[15] The findings herein demonstrate that purified PSA can induce a regulatory phenotype and IL-10 production in naïve T cells isolated from patients

with MS. Ongoing work in our laboratory has shown that the amplified IL-10 response is not genetically restricted across multiple human haplotypes (Kasper, LH, unpublished data). We found an increased frequency of T cells acquiring Foxp3 expression when exposed to PSA in cells isolated from healthy controls, untreated MS patients, and MS patients actively treated with GA. GA has been previously shown to increase the frequency of $Foxp3^+$ T cells and recover the suppressive capacity of T_{regs} in patients with MS.[22] In addition, treatment with PSA significantly increased the MFI of Foxp3 in both groups of MS patients but not healthy controls. $CD4^+CD25^+$ T_{regs} isolated from MS patients were found to have significantly reduced expression of Foxp3.[23] Since the level of Foxp3 expression can impact suppressive function *in vivo,*[24] the ability of PSA to increase the MFI of Foxp3 provides further evidence that PSA could potentially reverse the functional defects characterized in the T_{reg} population of MS patients. We also show that PSA can significantly increase the production of IL-10. Both $Foxp3^+$ and $Foxp3^-$ T_{regs} are capable of producing IL-10. In this ELISA-based analysis, we neither distinguish between the two populations nor identify the cellular source of IL-10, but previous work using outer membrane vesicles isolated from *B. fragilis* has shown that T cells, and not DCs, are responsible for IL-10 production following exposure to PSA.[25] The current study was limited by low numbers of naïve T cells isolated from individual MS patients, and future studies will require additional flow cytometric analyses such as CD25 and IL-7R staining to determine if the induced T_{regs} acquire a classical $CD25^{high}CD127^{neg/low}$ phenotype and intracellular cytokine detection of IL-10. *In vitro* suppression assays will also be important to confirm that PSA-induced T_{regs} can significantly reduce the proliferation of activated lymphocytes. Furthermore, while we have focused these studies on the ability of naïve T cells to convert to T_{regs}, others have shown that memory T cells also have the potential to acquire regulatory characteristics when appropriately stimulated.[26] Indeed, therapeutic strategies focused on the modulation of the gut microbiome to induce and/or enhance multiple subsets of regulatory cells could prove most effective in the case of MS.[27] Our findings suggest that novel gut microbiome-directed therapeutics such as PSA, possibly coupled with current FDA-approved immune-modulating drugs, could further enhance T_{reg}-mediated immunosuppression in the context of CNS autoimmunity.

Acknowledgments

The authors thank DartLab for their assistance coordinating the acquisition of samples from healthy subjects and the employees of the Neurology Associates and Concord Hospital Laboratory Services for their assistance coordinating the acquisition of samples from MS patients, especially Dr. Ann Cabot, Carol Fraser and Allyson Geary.

Author Contributions

LHK and SCB conceived and designed the study. JNB, ABP, and SCB acquired and analyzed data. JNB, LHK, and SCB wrote the manuscript.

Conflict of Interest

JNB, ABP, and SCB have nothing to report. LHK serves as a consultant and participates in the Teva Neuroscience speaker bureau. His laboratory has received funding from Teva to study the effects of GA on the gut microbiome.

References

1. 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.
2. Haas J, Hug A, Viehover A, et al. Reduced suppressive effect of CD4 + CD25high regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. Eur J Immunol 2005;35:3343–3352.
3. Astier AL, Meiffren G, Freeman S, Hafler DA. Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis. J Clin Investig 2006;116:3252–3257.
4. Miyake S, Kim S, Suda W, et al. Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to clostridia XIVa and IV clusters. PLoS ONE 2015;10:e0137429.
5. Chen J, Chia N, Kalari KR, et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. Sci Rep 2016;6:28484.
6. Jangi S, Gandhi R, Cox LM, et al. Alterations of the human gut microbiome in multiple sclerosis. Nat Commun 2016;7:12015.
7. Ivanov II, Frutos Rde L, Manel N, et al. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe 2008;16:337–349.
8. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci USA 2011;108(Suppl 1):4615–4622.
9. Ochoa-Reparaz J, Mielcarz DW, Ditrio LE, et al. Central nervous system demyelinating disease protection by the human commensal Bacteroides fragilis depends on polysaccharide A expression. J Immunol 2010;185:4101–4108.

10. Cree BA, Spencer CM, Varrin-Doyer M, et al. Gut microbiome analysis in neuromyelitis optica reveals overabundance of Clostridium perfringens. Ann Neurol 2016;80:443–447.
11. Wexler HM. Bacteroides: the good, the bad, and the nitty-gritty. Clin Microbiol Rev 2007;20:593–621.
12. Ochoa-Reparaz J, Mielcarz DW, Wang Y, et al. A polysaccharide from the human commensal bacteroides fragilis protects against CNS demyelinating disease. Mucosal Immunol 2010;3:487–495.
13. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 2008;453:620–625.
14. Round JL, Mazmanian SK. Inducible Foxp3 + regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci USA 2010;107:12204–12209.
15. Telesford KM, Yan W, Ochoa-Reparaz J, et al. A commensal symbiotic factor derived from bacteroides fragilis promotes human CD39(+)Foxp3(+) T cells and Treg function. Gut Microbes 2015;6: 234–242.
16. Singh V, Roth S, Llovera G, et al. Microbiota dysbiosis controls the neuroinflammatory response after stroke. J Neurosci 2016;13:7428–7440.
17. Macedo D, Filho AJ, Soares de Sousa CN, et al. Antidepressants, antimicrobials or both? Gut microbiota dysbiosis in depression and possible implications of the antimicrobial effects of antidepressant drugs for antidepressant effectiveness. J Affect Disord 2017;208: 22–32.
18. Hsiao EY, McBride SW, Hsien S, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 2013;155:1451–1463.
19. Kigerl KA, Hall JC, Wang L, et al. Gut dysbiosis impairs recovery after spinal cord injury. J Exp Med 2016;213:2603–2620.
20. Sampson TR, Debelius JW, Thron T, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of parkinson's disease. Cell 2016;167:1469e12–1469e80.
21. Dasgupta S, Erturk-Hasdemir D, Ochoa-Reparaz J, et al. Plasmacytoid dendritic cells mediate anti-inflammatory responses to a gut commensal molecule via both innate and adaptive mechanisms. Cell Host Microbe 2014;15:413–423.
22. Haas J, Korporal M, Balint B, et al. Glatiramer acetate improves regulatory T-cell function by expansion of naive CD4(+)CD25(+)FOXP3(+)CD31(+) T-cells in patients with multiple sclerosis. J Neuroimmunol 2009;216:113–117.
23. Huan J, Culbertson N, Spencer L, et al. Decreased FOXP3 levels in multiple sclerosis patients. J Neurosci Res 2005;81:45–52.
24. Chauhan SK, Saban DR, Lee HK, Dana R. Levels of Foxp3 in regulatory T cells reflect their functional status in transplantation. J Immunol 2009;182:148–153.
25. Shen Y, Giardino Torchia ML, Lawson GW, et al. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. Cell Host Microbe 2012;12:509–520.
26. Mohiuddin IH, Pillai V, Baughman EJ, et al. Induction of regulatory T-cells from memory T-cells is perturbed during acute exacerbation of multiple sclerosis. Clin Immunol 2016;166-167:12–18.
27. Takata K, Kinoshita M, Okuno T, et al. The lactic acid bacterium *Pediococcus acidilactici* suppresses autoimmune encephalomyelitis by inducing IL-10-producing regulatory T cells. PLoS ONE 2011;6:e27644.

