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

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 (Tregs). Importantly, studies have shown that while MS patients harbor normal frequencies of Tregs, their production of IL-10 and overall suppressive capacity is significantly reduced allowing for increased inflammation driven by effector cells. 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). 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. 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. In humans, Clostridium perfringens has been associated with the onset of neuromyelitis optica spectrum disorder, an MS-like disease.

B. fragilis is an anaerobic commensal that comprises 0.5–1.0% of the human colonic bacterial microflora in all mankind. 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 Tregs with IL-10-dependent immunosuppressive function. While it had previously been demonstrated that PSA acts via TLR2 to drive the induction of Tregs in mice, we have recently shown that PSA can induce the differentiation of Tregs when added to in vitro co-cultures of dendritic cells (DCs) and naive T cells isolated from the blood of healthy donors. In this study, we determined if PSA could similarly drive the differentiation of Tregs from naive 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). Naive 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% CO2. 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 naive T cells to Tregs 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). 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

|                  | Healthy controls | Untreated MS patients | GA-treated MS patients |
|------------------|------------------|-----------------------|------------------------|
| N (Sex)          | 18(F)            | 10(F)                 | 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. Naive T cells isolated from the indicated donors (healthy controls [HC/o], 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; *** P < 0.001. (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 Treg conversion in naive T cells isolated from patients undergoing treatment with GA (P = 0.0156). As an additional parameter to ascertain Treg induction, we measured the mean fluorescence intensity (MFI) of Foxp3 in the Foxp3+ Treg 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 Treg phenotype.

This result is of potentially important clinical value since Treg function is known to be impaired in patients with MS.4–7 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 naive 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 Treg function during MS. Furthermore, the observed trend would suggest that upregulation of IL-10 by PSA may amplify Treg conversion in combination with established MS therapeutics.

**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, Parkinson’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+ Tregs.13 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...
we have focused these studies on the ability of na proliferation of activated lymphocytes. Furthermore, while cells to convert to Tregs, others have shown that memory 10, but previous work using outer membrane vesicles iso-
this ELISA-based analysis, we neither distinguish between 
to PSA.25 The current study was limited by low numbers 
of naive 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 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 Tregs in 
patients with MS.22 In addition, treatment with PSA sig-
nificantly increased the MFI of Foxp3 in both groups of 
MS patients but not healthy controls. CD4+CD25+ Tregs 
isolated from MS patients were found to have significa-
cantly 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 Treg population of MS patients. We also show that PSA can significantly increase the production of IL-10. Both Foxp3+ and Foxp3– Tregs 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 iso-
lated 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 
naive 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 Tregs acquire a classical CD25highCD127neg/low phenotype and intracellular cytokine detection of IL-10. In vitro suppression assays will also be important to con-
firm that PSA-induced Tregs can significantly reduce the 
proliferation of activated lymphocytes. Furthermore, while 
we have focused these studies on the ability of naive T 
cells to convert to Tregs, others have shown that memory 
T cells also have the potential to acquire regulatory char-
acteristics when appropriately stimulated.26 Indeed, therapeu-
tic 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 Treg-mediated immunosuppression in the context of CNS autoimmunity.

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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 Invest 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-Repaz J, Miclcarz 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, Lloversa G, et al. Microbiota dysbiosis controls the neuroinflammatory response after stroke. J Neurosci 2016;36:12446–12457.

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 anti-microbial 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;166:167–172.

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.

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