Probiotic VSL_3 Induced Changes in the Gut Microbiome and Peripheral Immune Response in Multiple Sclerosis Patients

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Abstract

None of the disease-modifying therapies (DMTs) currently being used for the management of multiple sclerosis (MS) are 100% effective. In addition, side effects associated with the use of these DMTs have limited the practice of combination therapy. Hence, there is a need for safe immunomodulatory agents to fine-tune the management of MS. Probiotics represent an oral, nontoxic immunomodulatory agent that could be used in combination with current MS therapy. We designed a pilot study to investigate the effect of VSL3 on peripheral immune system function in MS patients. VSL3 administration induced an anti-inflammatory peripheral immune response characterized by decreased frequency of pro-inflammatory monocytes and decreased mean fluorescence intensity of HLA-DR on dendritic cells.

Glossary of Abbreviations
DMT: Disease-Modifying Therapies
EAE: Experimental Autoimmune Encephalomyelitis
FACS: Fluorescence-Activated Cell Sorting
MS: Multiple Sclerosis
PBMCs: Peripheral Blood Mononuclear Cells
SFB: Segmented Filamentous Bacteria

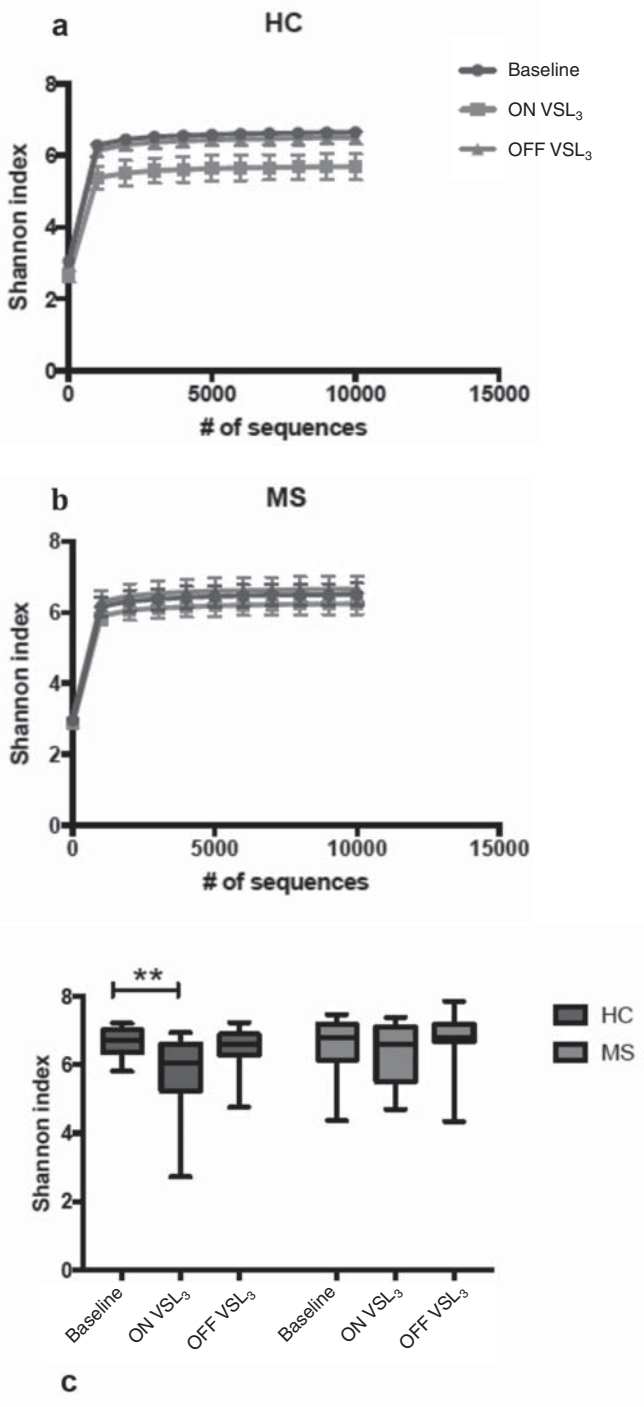
Material and Methods:

MS subjects (N = 9) and controls (N = 13) were orally administered VSL3 double strength sachets twice daily (total 3,600 billion CFU/day) for two months. VSL3 is a probiotic mixture containing 3 x 1011/g of viable lyophilized bacteria including three strains of bifidobacteria (*Bifidobacterium longum, Bifidobacterium infantis*, and *Bifidobacterium breve*), four strains of lactobacilli (*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii subsp. bulgaricus*, and *L. plantarum*), and one strain of streptococcus (*S. salivarius subsp. thermophilus*). Stool specimens were collected prior to, at discontinuation of therapy, and three months thereafter. Frozen peripheral blood mononuclear cells (PBMCs) were used for fluorescence-activated cell sorting (FACS), and stool samples were used for 16S profiling by Illumina MiSeq.

Introduction

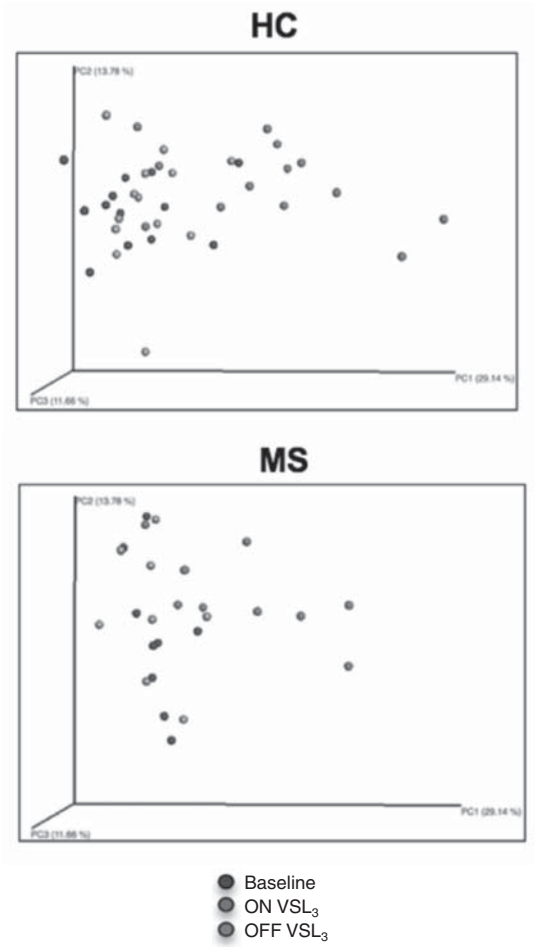
The gut microbiome has been implicated in several autoimmune disorders including inflammatory bowel disease, rheumatoid arthritis, and MS.[1-4] Studies in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, have shown that perturbation of the composition of the gut microbiota affects mice susceptibility to develop EAE.[5,6] Lloyd Kasper and colleagues have shown that colonization of mice with bacteroides fragilis ameliorates EAE.[7] Sarkis Mazmanian and colleagues have reported that colonization of mice with segmented filamentous bacteria (SFB), a Th17 inducer, exacerbates EAE.[8] We have reported alteration in the gut microbiome of MS patients[1] (see Appendix on page 87). Several other groups have also reported dysbiosis in the gut microbiome of MS subjects.[3,4] These findings suggest that manipulation of the gut microbiome by the use of probiotics, for example, could potentially benefit MS patients. Probiotic VSL3 is a cocktail of eight bacteria that has a good safety profile. Probiotic VSL3 has been shown to induce IL-10+ and IL-10-dependent TGF-β-bearing regulatory cells in the gut of a mouse model of colitis.[9] Furthermore, VSL3 has been shown to promote an anti-inflammatory response in the gut of a mouse model of peanut allergy and diabetes.[10,11] VSL3 immunomodulatory properties extend to the central nervous system. For example, VSL3 has been shown to promote neuroprotection in a mouse model of traumatic spinal cord injury.[12] Another study reports decreased microglial cell activation as well as CNS monocytes infiltration leading to sickness behavior in a mouse model of liver inflammation following VSL3 adminstration.[13] In humans, VSL3 has been shown to benefit patients with pouchitis, ulcerative colitis, and diabetes.[14-16] Very little is known about the effect of VSL3 on peripheral immune function in humans. Hence, the goal

Figure 1. Probiotic VSL_3 effect on alpha diversity of the gut microbiome. Rarefaction curves were calculated at multiple sequence depths on MiSeq platform for Shannon entropy to compare differences in alpha diversity at the indicated time points in a) controls (n=13) and b) MS patients (n=9). Shannon index was also measured at a depth of 10000 reads in c) controls and MS subjects at the indicated time points.



* P<0.05
** P<0.01

Figure 2. Probiotic VSL_3 effect on microbial community structure. Principal coordinate analysis of weighted Unifrac distances colored according to time points in healthy controls (HC) and multiple sclerosis patients (MS).



of this study is to investigate the effect of probiotic VSL3 on peripheral immune function in healthy controls and MS subjects.

Administration of Probiotic VSL_3 Is Associated with Changes in the Structure and Composition of the Gut Microbiome in Healthy Controls and and MS Patients

Microbial DNA was extracted from fecal samples, and 16S rRNA gene sequencing was performed on Illumina MiSeq platforms using primers targeting the V4 variable region. To assess overall differences in microbial community structure in MS patients and controls, we calculated measures of alpha and beta diversity. Alpha diversity represents microbial diversity within each sample, whereas beta diversity measures differences between samples. Shannon entropy, an alpha-diversity measurement of richness and evenness was measured at multiple sequencing depths using rarefaction curves. We found that administration of VSL3 was associated with decreased alpha diversity in healthy controls (Figure 1a and 1c). No change in alpha diversity was observed in MS subjects following VSL3 administration (Figure 1b).

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Figure 3. Compositional differences in fecal microbiota before and after VSL_3 administration in healthy controls (HC) and multiple sclerosis patients (MS).

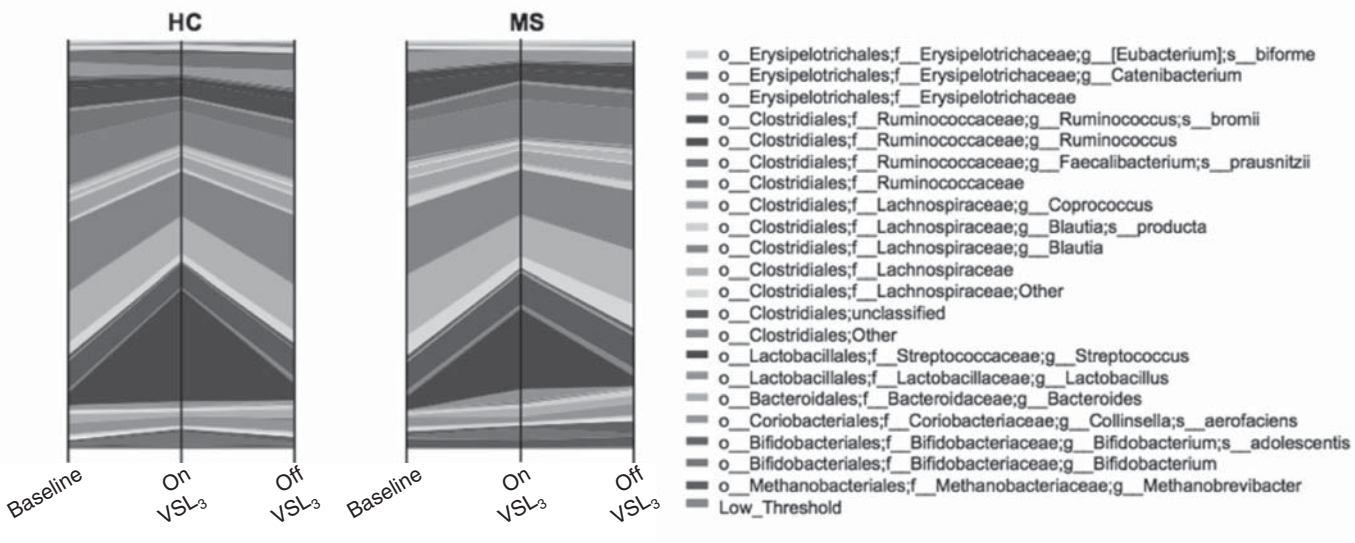
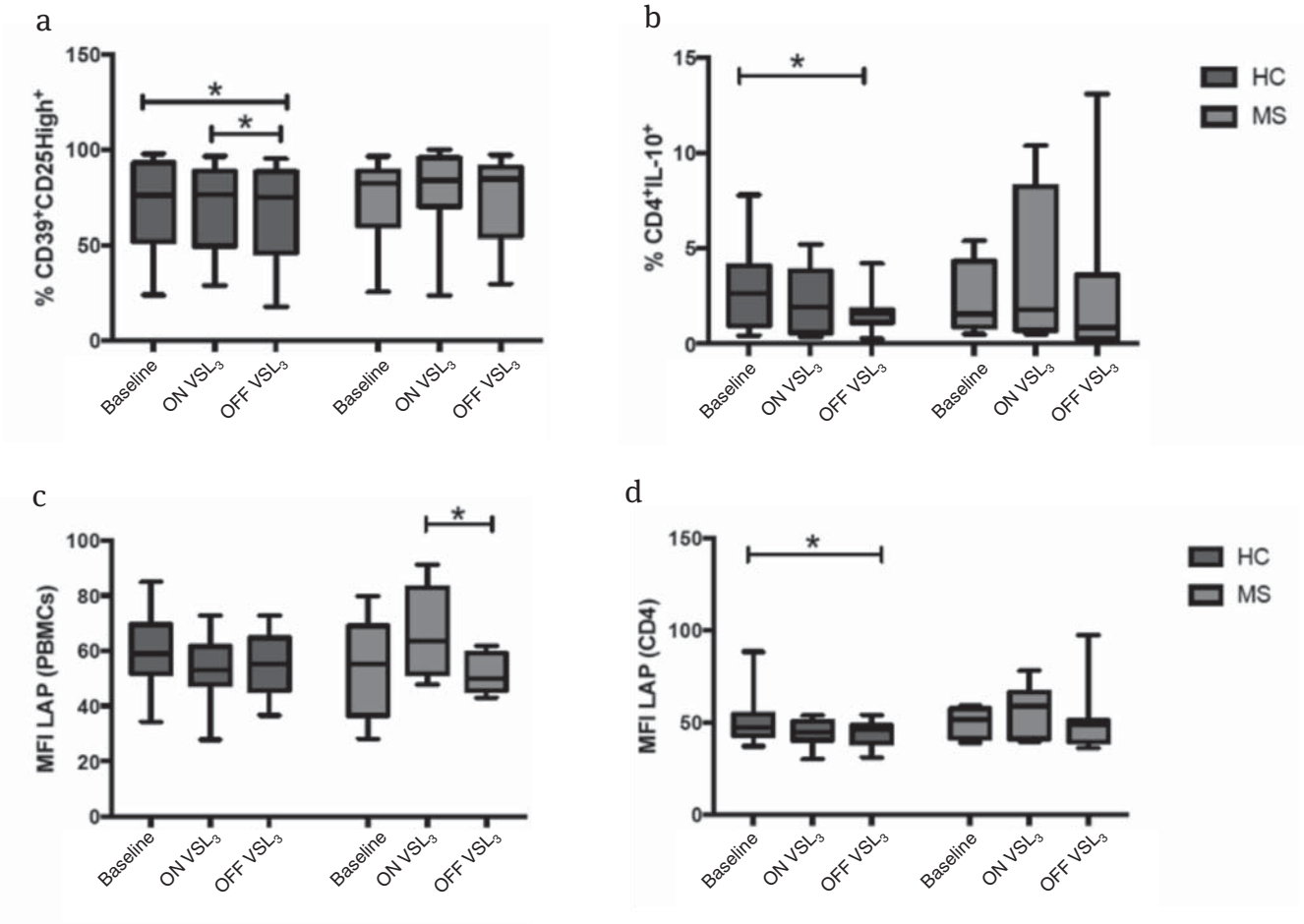
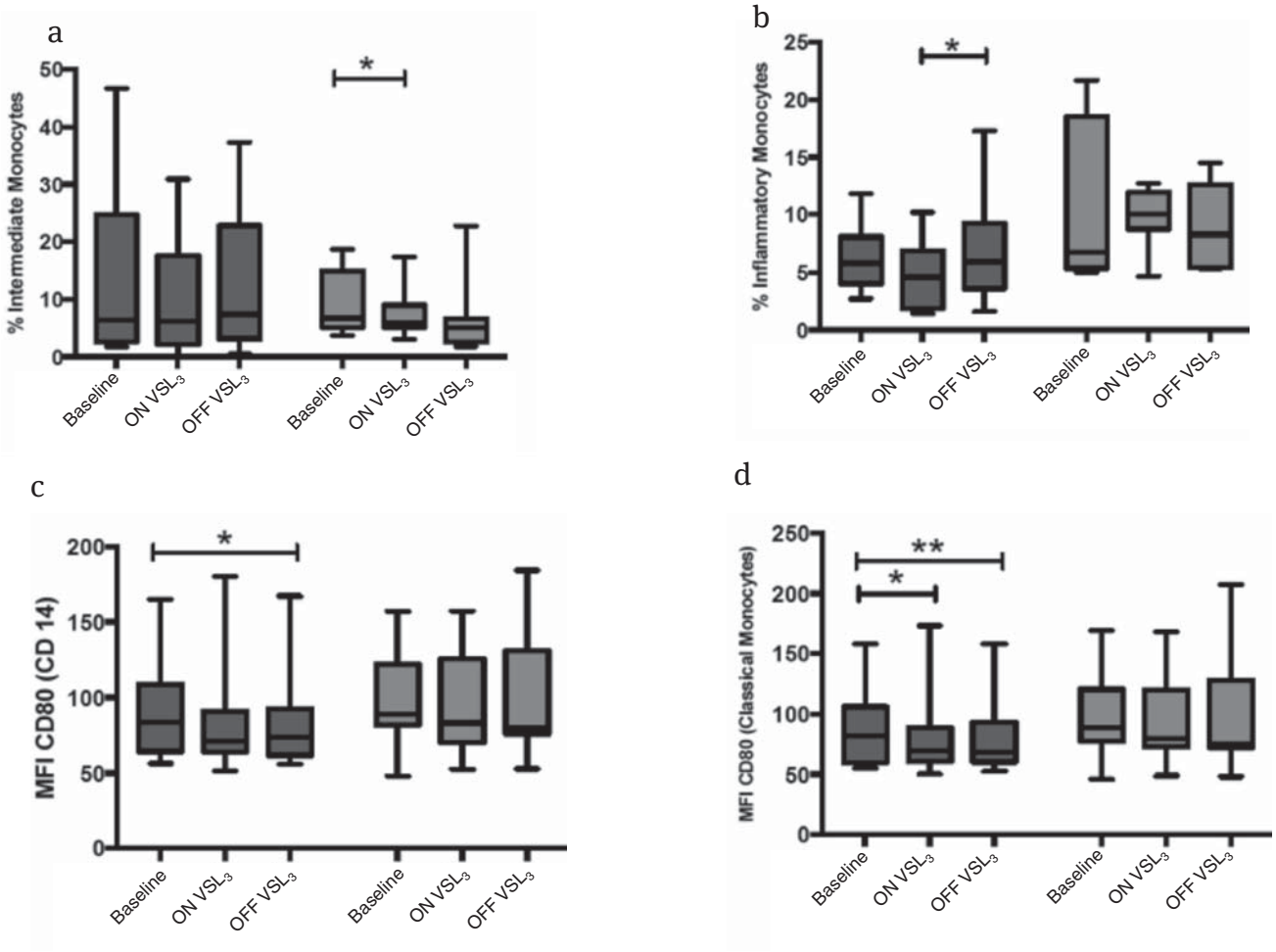


Figure 4. Probiotic VSL_3 effect on mean fluorescence intensity (MFI) of latency associated peptide (LAP) and T regulatory cells frequency. FACS analysis was used to compare (a) the MFI of LAP on CD4 T cells and (b) total LAP MFI in healthy controls (HC) and multiple sclerosis patients (MS) at the indicated time points. FACS analysis was used to compare (c) the frequencies of CD4+ IL-10+ T cells and (d) CD39+ CD25 high T cells in healthy controls (HC) and multiple sclerosis patients (MS) at the indicated time points.



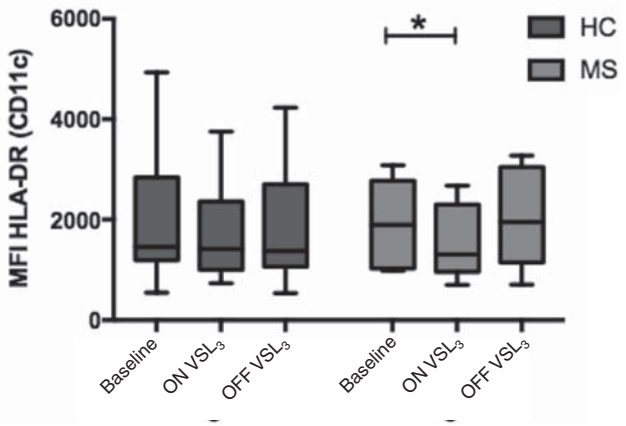
* P<0.05

Figure 5. Probiotic VSL_3 effect on pro-inflammatory monocytes frequency and mean fluorescence intensity (MFI) of CD80. FACS analysis was used to compare (a) the frequencies of intermediate monocytes and (b) inflammatory monocytes in healthy controls (HC) and multiple sclerosis patients (MS) at the indicated time points. FACS analysis was used to compare CD 80 MFI on (c) total monocytes and (d) classical monocytes in healthy (HC) and multiple sclerosis (MS) patients at the indicated time points.



* P<0.05; ** P<0.01.

Figure 6. Probiotic VSL_3 effect on mean fluorescence intensity (MFI) of HLA-DR on dendritic cells. FACS analysis was used to compare the HLA-DR MFI on dendritic cells from healthy control (HC) and multiple sclerosis (MS) patients at the indicated time points.



* P<0.05

Following administration of probiotic VSL3, communities shifted out along PC1 and then shifted back to baseline distribution on discontinuation of VSL3 (Figure 2). We next investigated whether the relative abundance of specific bacterial strains differed following administration and discontinuation of VSL3 in controls and MS subjects (Figure 3). The relative abundance of genera Streptococcus and Blautia, as well as order clostridiales and family lachnospiraceae, all belonging to the phylum Firmicutes were increased following administration of VSL3. The relative abundance of these bacterial strains returned to baseline following discontinuation of VSL3 (Figure 3).

VSL_3 Effect on Peripheral Immune Function

Blood samples from all subjects were collected at baseline, two months after initiation of VSL3, and at three months post discontinuation of VSL3. PBMCs were isolated from these blood samples at all three time points and subsequently used for FACS analysis. Given prior reports of increased LAP+

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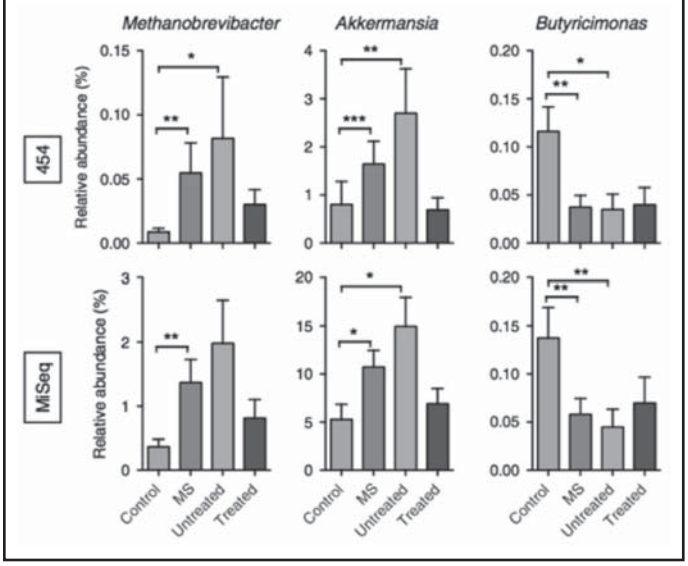
Appendix. Relative abundance of genera in the fecal microbiota that are significantly altered between healthy controls (n=43) and MS patients (n=60) or between untreated (n=28) and treated MS patients (n=32) as analyzed by two independent sequencing platforms.



and IL10+ T regulatory cells (T regs) in the gut following VSL3 administration, we conducted experiments to investigate the effect of probiotic VSL3 on peripheral T regs. No significant change in the relative frequency of IL-10+ or CD39+ T regs as observed following administration of VSL3. Discontinuation of VSL3 was associated with decreased relative frequency of IL-10+ as well as CD39+ T regs was observed in healthy controls (Figure 4a and 4b).We also observed decreased mean fluorescence intensity of LAP following discontinuation of VSL3 in healthy controls and MS patients (Figure 4c and 4d). We also did immune-cell profiling of monocytes from healthy control and MS subjects at the three time points and found that administration of VSL3 was associated with decreased frequency of intermediate monocytes in MS patients (Figure 5a). Discontinuation of VSL3 was associated with increased frequency of inflammatory monocytes in healthy control (Figure 5b). In healthy controls, we also observed decreased mean fluorescence intensity of costimulatory marker CD80 on monocytes following administration of VSL3 (Figure 5c and 5d). In MS patients, we observed decreased mean fluorescence intensity of HLA-DR on dendritic cells following administration of VSL3.

Conclusion

Our studies showed that administration of probiotic VSL3 was associated with an increased relative abundance of bacterial strains belonging to the phylum Firmicutes, such as Streptococcus and Blautia in both HC and MS patients. At the immune level, VSL3 effect was predominantly seen on monocytes and dendritic cells. VSL3 administration induced an anti-inflammatory peripheral innate immune response characterized by decreased frequency of intermediate monocytes in MS subjects as well as decreased mean fluorescence intensity of costimulatory marker CD80 on monocytes in controls. Administration of VSL3 was also associated with decreased mean fluorescence intensity of HLA-DR on dendritic cells in MS subjects. On the other hand, discontinuation of VSL3 induced a pro-inflammatory immune response characterized by increased frequency of inflammatory monocytes in controls. We also observed decreased frequency of IL-10+ and CD39+ T regs in controls as well as decreased mean fluorescence intensity of LAP in both healthy control and MS subjects following discontinuation of VSL3.

Taken together, these findings suggest that the use of the probiotic VSL3 can induce an anti-inflammatory peripheral innate immune response in MS subjects. Further studies are needed to validate these findings and to determine if VSL3 induced anti-inflammatory peripheral immune response is associated with improved disease outcome in MS patients.

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