Complex Interplay of Forkhead BoxP3, Interleukin 22, and Interleukin 17 in Multiple Sclerosis Patients on Disease Modifying Therapy

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Introduction

Multiple Sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system. The imbalance in the immune system leads to autoantigen recognition and the destruction of myelin, an insulating sheath of neurons utilized to increase the speed and efficiency of electrical impulses. Several predisposing factors including genetics, viral infections, climate, and age are correlated but a clear association has yet to be identified.[1,2,3,4] The illness course varies depending on underlying inflammation, influenced by the balance of regulatory and effector immune cells and their respective cytokines.[5] Understanding the complex interplay of the cytokine levels and transcription factors in MS patients can potentially help identify response to therapy and track the course of illness. Our lab measured Interleukin 22 (IL-22), a cytokine with regulatory and inflammatory activity, Forkhead Box P3 (FoxP3), a regulatory T cell transcription factor, and Interleukin 17 (IL-17), an inflammatory cytokine. Previous studies indicate elevation of IL-22 and IL-17 and lower FOXP3 levels in MS patients relatively. [6,7,8,9] However, the levels of IL-22, IL-17, and FOXP3 have not been investigated in patients on Disease Modifying Therapy (DMT). Elucidating the impact of DMT on cytokine regulation could modifying current treatments to be patient-specific by identifying and tracking inflammatory cells and their markers and help identify other modalities of treatment. We also utilized TGF Beta, a cytokine with neuroprotective function,[10] IL-22, and IL-17, as potential co-therapies in MS samples to identify their utility as potential treatment modalities.

Further, we also investigated levels of T helper 40 cells (Th40), a subset of CD4+ T cells expressing the CD40 receptor, immune cells upregulated in autoimmune diseases such as MS and Experimental Autoimmune Encephalomyelitis (EAE) model, the accepted model for MS in mice.[11,12,13] Th40 levels have not been previously studied in patients on DMT and can help serve as an alternative inflammatory marker to trac illness course.

Materials and methods

Samples were collected from consented patients seen at the The University of Colorado Department of Neurology Multiple Sclerosis Clinic, The University of Colorado Department of Neurology Headache Clinic, and The University of Colorado Hospital. Patients were diagnosed with MS or without any know autoimmune neurological disease (Control). MS patients were on DMT (Interferon beta 1a, Natalizumab, and Dimethyl fumarate) with out recent flares of illness. Lymphocytes were isolated by Ficol-Hypaque and stimulated. Cells were stained for CD3, CD4, IL-17, IL-22, and FoxP3 and treated with IL-22, IL-17, or TGF Beta. Flow cytometry was conducted using a MACSQuant Analyzer. The gates were set such that the upper left/right and lower right quadrants had less than 1% of events. Data was analyzed using GraphPad Prism.

Results

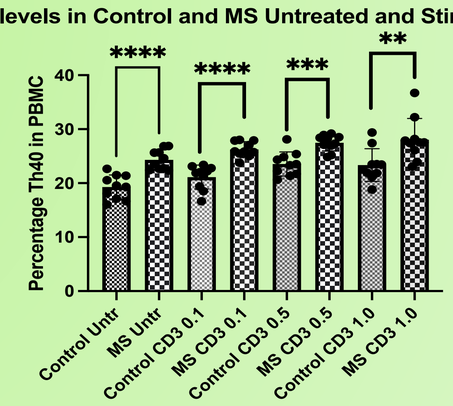

Figure 1. Peripheral blood mononuclear cells (PBMCs) were collected from MS patients stable on disease-modifying therapy (n=4) and control subjects (n=5). Samples were untreated or stimulated with CD3 0.1, 0.5, or 1.0µg. Th40 cell levels in MS samples on DMT were compared to control samples across multiple groups based on concentrations of CD3 utilized. Th40 cells, effector immune cells with inflammatory activity, remain statistically higher in MS samples despite DMT. Increasing CD3 concentrations correlated to increased Th40 levels in MS samples but without statistical significance. Statistical analysis revealed a significant overall difference between samples ($p < 0.0001$) as determined by ANOVA. Further comparisons between MS and control samples were conducted using two-tailed, unpaired t-tests.

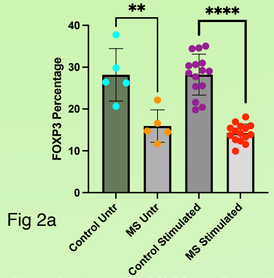

Fig 2a

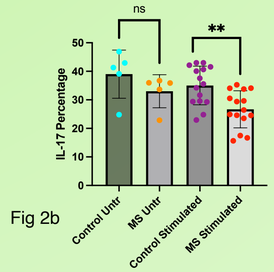

Fig 2b

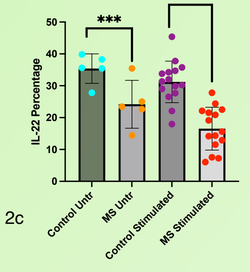

Fig 2c

Figure. 2. Comparing FOXP3, IL-17, and IL-22 levels in PBMCs of MS samples (n=4) relative to control (n=5). Two-tailed, unpaired t-tests was conducted to determine differences between samples. A) FOXP3 levels were assessed in PBMCs from MS patients stable on DMT and compared to controls. Both set of samples were untreated or stimulated. FOXP3 levels were statistically lower in untreated and stimulated MS samples relative to control. This correlates to lower regulatory immune cell function in MS samples. B) IL-17 levels were assessed in PBMCs from MS patients stable on DMT and compared to controls. Both set of samples were untreated or stimulated. IL-17 levels were lower in untreated and stimulated MS Samples, without and with statistical significance, respectively. This can be correlated to suppressed IL-17 inflammatory activity due to DMT. C) IL-22 levels were assessed in PBMCs from MS patients stable on DMT and compared to controls. Both set of samples were untreated or stimulated. IL-22 levels were statistically lower in untreated and stimulated MS samples relative to control. IL-22 levels decrease with stimulation in control and MS samples relative to untreated samples without and with statistical significance, respectively. Relatively lower IL-22 levels in MS samples correlate with suppressed inflammatory activity likely due to DMT.

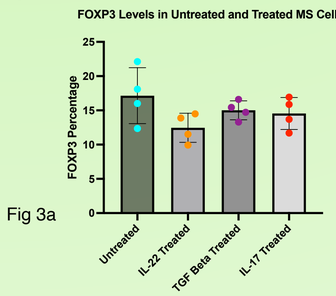

Fig 3a

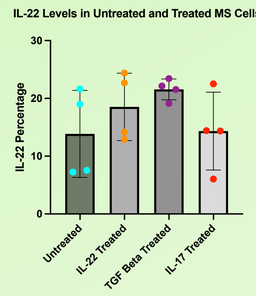

Fig 3b

Figure 3. FOXP3 and IL-22 levels in PBMCs of samples collected from MS patients stable on DMT (n=4) treated with IL-22, TGF Beta, or IL-17 relative to untreated samples. A) FOXP3 levels in PBMCs collected from MS samples treated with IL-22, TGF Beta, or IL-17, and compared to untreated samples. Statistical analysis was conducted to determine differences in FOXP3 levels between samples (P=0.1568). Untreated samples had higher FOXP3 levels relative to treatment groups. TGF Beta, a primarily regulatory cytokine, had the highest level of FOXP3 levels compared to other treatments. B) IL-22 levels were assessed in PBMCs collected from MS samples treated with IL-22, TGF Beta, or IL-17, and compared to untreated samples. Statistical analysis was conducted to determine differences in IL-22 levels between samples (P=0.2576). IL-22 levels were lower in untreated group relative to treatment groups with higher IL-22 levels observed in TGF Beta treated samples relative to other treatment groups.

Conclusions

Th40 cells, a subset of effector immune cells in MS and EAE, a primary driver of autoimmune diseases, remains elevated in MS patient on DMT. MS samples had higher Th40 levels in untreated cells, thus they had higher baseline Th40 levels relative to control. Th40 cells remain elevated in MS samples when stimulated relative to control samples (Fig 1). Further, Th40 levels increased with stimulation in MS samples as opposed to IL-17 (Fig 2b) and IL-22 (Fig 2c) levels, pro-inflammatory cytokines in MS, which were relatively lower in MS samples than controls and decrease with stimulation. Thus, the DMT did not have regulatory activities on Th40. Broadening DMT targets to include Th40 or utilizing additional therapies to target Th40 cells can help control and reduce underlying inflammation.[14,15]

FOXP3 levels, the primary transcription factor of T regulatory cells, were lower in MS samples (Fig 2a). DMTs primarily target effector immune cells resulting in adverse immunosuppression.[16,17] Lower FOXP3 levels can be utilized as biomarkers for tracking regulatory cell activity and targeted for therapy. In addition, a shift in focus to increasing immune regulatory cell activity should be explored in addition to regulating effector immune cell function.

TGF Beta, a context dependent cytokine with primarily anti-inflammatory activity, treated MS samples had high FOXP3 (Fig 3a) and IL-22 (Fig 3b) levels relative to IL-22 and IL-17 treated cells. The difference observed were not statistically significant and FOXP3 levels were higher in untreated samples. Thus, targeting TGF Beta might not be a suitable DMT candidate. However, further studies are required to elucidate the complex role of TGF Beta in MS.

References

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Future Directions

Increasing sample size and including MS patients prior to starting DMT can help understand the impact of DMT on effector T Cells such as Th40 cells and anti-inflammatory cytokines as well as regulatory T cells and anti-inflammatory cytokines.

Inflammatory markers are a snapshot in the course of illness. Investing in tracking these markers across time and correlating the underlying physiology to physical manifestation can aid in clinical decision making.

Adjusting the concentrations of TGF Beta in subsequent studies can help evaluate the utility of exploring TGF Beta as a potential DMT.

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For further information

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