Using Th40:Treg Ratio as a Predictor of Multiple Sclerosis and Other Autoimmune Diseases
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INTRODUCTION
What is Multiple Sclerosis (MS)?
• Autoimmune disease that results in the demyelination of nerves; impedes transmission of messages1
• Symptoms: vision problems (tunnel vision); numbness/weakness of the limbs; coordination problems, especially in gait; paraparesis2
• Cause: unknown, autoimmunity
• McDonald Criteria: Tool used to diagnose MS1,2
  Main requirement- have 2 attacks or 2 white matter lesions disseminated in time and space. The presence of lesions is determined through MRI.3
  Goal is to eliminate other diseases that show symptoms similar to MS; MS has to be the last possible explanation
• Based on HLA specificity: certain HLA haplotypes are associated with disease. In MS, HLA-DR2 and HLA-DQ6 are associated with the disease5

Th40 & T regulatory (Treg) cells
• Th40: Helper T-cell subpopulation. Defined by CD3+/CD4+/CD40+
• Elevated in autoimmune diseases, including Type 1 Diabetes (T1D)6
• Treg: help suppress inflammation/immune response by inhibiting proliferation and cytokine production in effector cells.3 Defined by CD3+/CD4+/CD25+/CD40-
• Treg cells usually occur at the same level as Th40 cells in individuals without autoimmune disease. But in autoimmunity they are at a lower level and don’t catch up or regulate to a normal equivalent activity6

PURPOSE
• Hypothesis: Comparing Th40 to Treg levels, we expected to see that MS and T1D would have a higher level of Th40 to Treg, thus limiting the regulation of Th40 proliferation. This ratio can be used to better predict autoimmunity in some disease models.
• The development of a test using these ratios to predict the likelihood of a patient developing MS or other autoimmune disease would be highly likely and could help create a better predictive tool.
• Better, earlier diagnosis, hopefully leading to earlier interventions, and thus limiting symptomology.
• Save patients time and money, allowing for greater treatment options.

MATERIALS & METHODS
1. Staining of lymphocytes
   • Blood samples were obtained from MS patients and from individuals without autoimmune disease. Patient scheduling was facilitated by the Barbara Davis Center.
   • Ficoll separated PBMCs
   • Washed with RBC lysis buffer
   • Stained with antibodies to CD4, CD40, CD3, CD25, CD45 r/o and CD8
   • Rinsed with PBS
   • Fixed in paraformaldehyde 4%
   • Part of the fixed cells were permeabilized with eBioscience Permeabilization Buffer and internally stained for FXP3.
2. Ran stained samples on flow cytometer
3. Evaluated results from flow cytometer in FlowJo

RESULTS

CONCLUSIONS
• Th40 levels were elevated in T1D. This seems to hold true in MS patients as well.8
• In T1D the levels of Tregs are lower and appear to have limited ability to regulate the immunoreponse. This appears to be similar in MS.
• It was noted that the Treg population was also limited in some of the control patients. This might be due to exposures to illness or other external factors.8

FUTURE DIRECTIONS
• Observe the effects of increasing the number of Treg cells so that they are at the same level as activated Th40 cells –does it result in the quiescence of Th40 activity or in any changes in disease course?
• Use Th40:Treg ratio as a predictor of other autoimmune diseases
• Develop a patient database that will allow for comparison of other external factors, as well as Th40 and Treg populations. This can assist in the development of a more concrete picture.

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REFERENCES