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Research grant – progress report

Influence of the short-chain fatty acid propionic acid on the peripheral immune regulation in the context of chronic inflammatory demyelinating polyneuropathy (CIDP)

Grant Award Amount: $ 197.306 Project Duration: July 2021–July 2023, extended

Principal investigators:

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Grant Summary

The intestinal immune system and the community of intestinal microbes, the microbiome, determine autoimmune inflammation. Nutrition is a major influence factor on microbiome and supplementation of the fatty acid propionic acid showed positive immunomodulatory effects in diseases like multiple sclerosis. The aim of this project is to investigate the immunomodulatory effect of propionic acid in patients with chronic inflammatory demyelinating polyneuropathy (CIDP), who take propionic acid orally for 90 days. Immunomodulatory effects shall be detected in inflammatory cell populations and in microbiome analyses of stool samples.

Study endpoints:

Before and after 3 months treatment with PA, as well as after 3 months without treatment the following parameters are evaluated:

- Clinical outcome using disability and symptom scores
- Peripheral blood: quantification of SCFA, immune cell phenotyping
- Stool samples: Microbiome analyses (next generation sequencing)

Milestones achieved

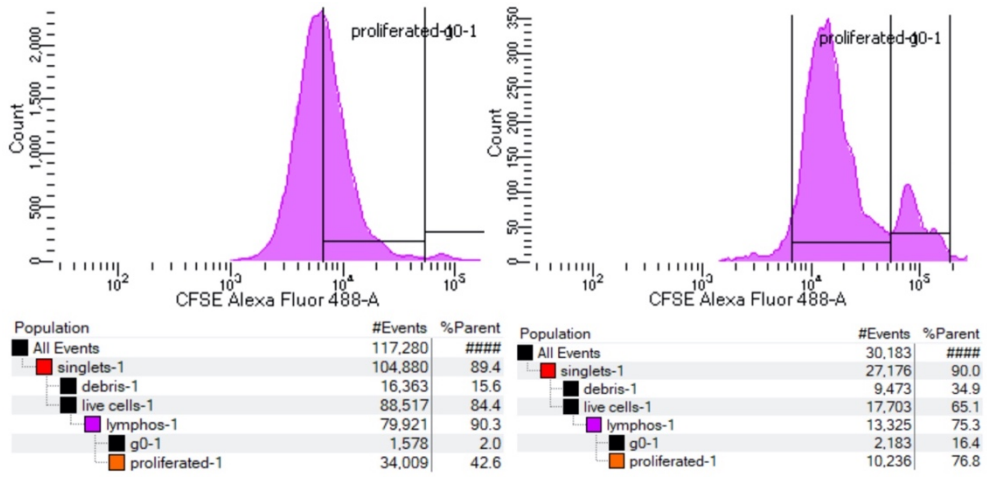
- Establishing and testing the immunological assays in our laboratory was completed in January 2022, two medical PhD students were trained in 2021 to support the study personnel. The method is described in the methods section.
- The first patient with CIDP was enrolled on 02/14/2022.
- Currently, 49 of 60 patients and 18 of 30 controls are enrolled in the study.
- We performed an interim analysis after 38 patients and 15 controls, the results can be seen in the results part.

Methods

T-cell differentiation under the influence of PA

Immunophenotyping was used to analyze the T cell composition. For this purpose, a whole blood sample was stained with specific antibodies and analyzed for different T cell populations using flow cytometry. In addition to the CD3+, CD4+ and CD8+ cells, the T helper cells TH1-, TH2-, TH17-cells and Tregs, as well as the cytotoxic T cells TC1- and TC2-cells were quantified. A suppression assay was also performed to determine the suppressive capacity of the Tregs. For this purpose, Tregs isolated from a whole blood sample were placed in culture with the other lymphocytes, which were stained with the intracellular dye carboxyfluorescein succinimidyl ester (CFSE). In addition, there was a control condition in which unstained lymphocytes were placed in culture together with stained lymphocytes. These cells were incubated for four days at 38° C and 5% CO2. On the fourth day, the cell proliferation rate of the stained cells was quantified by flow cytometry.

The graphs below show the proliferation of lymphocytes after four days in culture without (left) and with Tregs (right). In the condition without Tregs, 79,921 lymphocytes are present, in the Treg condition only 13,325, although the same number of lymphocytes were added to both conditions on day 0.



Population	#Events	%Parent
All Events	117,280	####
singlets-1	104,880	89.4
debris-1	16,363	15.6
live cells-1	88,517	84.4
lymphos-1	79,921	90.3
g0-1	1,578	2.0
proliferated-1	34,009	42.6

Population	#Events	%Parent
All Events	30,183	####
singlets-1	27,176	90.0
debris-1	9,473	34.9
live cells-1	17,703	65.1
lymphos-1	13,325	75.3
g0-1	2,183	16.4
proliferated-1	10,236	76.8

Results

T-cell differentiation under the influence of PA – results of the interim analysis

We performed T-cell differentiation assays as interim analysis with results of 38 patients and 15 controls. CIDP patients had a significantly lower proportion of TH1 cells ($p < 0.05$), a lower proportion of Tregs ($p < 0.05$) and a lower Treg/TH17 ratio ($p < 0.05$) at baseline compared to healthy control subjects (see figure below). The other T-cell populations showed no significant differences between CIDP subjects and healthy control subjects. After taking 500 mg PA twice daily for 90 days, there was no change in the composition of the immune cells. The suppressive capacity of the Tregs of the CIDP patients showed no significant difference to the healthy control subjects either at baseline (see figure below) or after taking PA.

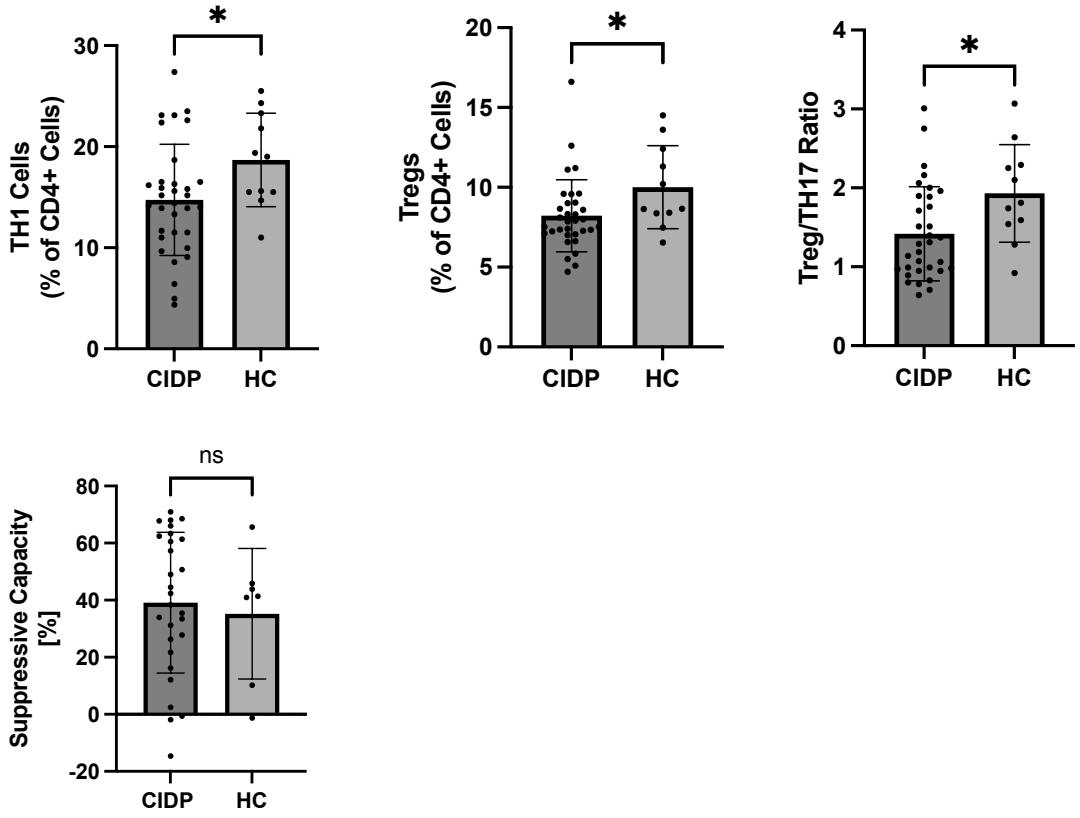


Figure: Baseline results of T cell analysis and suppressive capacity of CIDP patients and healthy controls

Changes to the original proposal

Instead of treating CIDP patients with PA for 3 months, we decided to treat the remaining patients and controls for only 30 days to see effects on the immune cells which might arise only temporarily and for a shorter time in blood, as seen in the literature in other diseases [1,2].

Open tasks and challenges

- Inclusion of remaining patients and controls.
- SCFA and microbiome analysis en bloc after completion of all patients and controls in cooperation with Prof. Dr. Gabriele Stangl, Institute of Agricultural and Nutritional Sciences, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany, in cooperation with Prof. Dr. Katrin Marcus, Center for Protein Diagnostics, Ruhr-University Bochum, Bochum, Germany, in cooperation with Dr. Sabrina Mühlen, Molecular Immunology, Ruhr-University Bochum, Bochum, Germany and in cooperation with Dr. Lajos Marko/Dr. Sofia Forslund, Max Dellbrück Center Berlin, Berlin, Germany.

Finance

Costs to date:

Personnel	**33.075 Euro**
Direct expenses:	
Materials and supplies	**62.013 Euro**
Total	**95.088 Euro / 102.947 $**
Remaining funding amount	**94.359 $**

References:

[1] Duscha A, Gisevius B, Hirschberg S, et al. Propionic Acid Shapes the Multiple Sclerosis Disease Course by an Immunomodulatory Mechanism. Cell 2020; 180: 1067-1080.e16. doi:10.1016/j.cell.2020.02.035

[2] Meyer F, Seibert FS, Nienen M, et al. Propionate supplementation promotes the expansion of peripheral regulatory T-Cells in patients with end-stage renal disease. J Nephrol 2020; 33: 817–827. doi:10.1007/s40620-019-00694-z