

The Role of the Gut Microbiome in the Pathogenesis of ITP

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The role of the microbiome in the digestive tract has become the focus of a lot of research in various autoimmune diseases, including in ITP. This study was designed to define the contribution of an imbalanced digestive system (particularly the gut microbiome) on both B cell-mediated and T cell-mediated (immune cells) ITP and to develop a predictive model of illness severity.

In a subset group of patients with ITP, an auto-reactive T cell-mediated process (immune cell process leading to the development of antiplatelet antibodies) is thought to contribute to platelet destruction. The triggering events for ITP are still poorly understood but thought to be mostly related to antigen exposure via viral infection or immunization. These exposures may initiate gut microbial imbalance through inflammation and pathogenic (disease-causing) microbe overgrowth. The contribution of gut microbiome disruptions to autoimmune hematologic disorders, and ITP, have not previously been investigated.

Imbalance of the gut microbiome however has been shown to lead to disease in other human autoimmune diseases. This overgrowth correlated with increased numbers of pathogenic T_H17 cells (helper T cells) correlated with autoimmune diseases and reduction in regulatory T-cell populations. Gut microbial alterations have also been identified at diagnosis in Type I Diabetes Mellitus. Thus, it is thought that disturbances of the gut microbiome may contribute to the underlying mechanisms of autoimmune cytopenia (when one of your blood cell types are in abnormal quantity) by priming for auto-reactive disease.

This study analyzed the gut microbiome populations of 8 pediatric patients with newly diagnosed severe acquired aplastic anemia (representing a T-cell mediated autoimmune destruction of hematopoietic stem and progenitor cells) and 10 pediatric patients with newly diagnosed ITP. There were 6 males and 2 females in the aplastic anemia group and 5 males and 5 females in the ITP group. The median age (middle of age range) of patients in the aplastic anemia cohort was 10 years (range: 6-19 years) while the median age of ITP patients was 7.5 years (range: 14 months to 14 years). Matched healthy controls were included in the study. We utilized 'shotgun sequencing' of gut microbial populations which is a method of determining the composition of the gut microbiome and comparing to healthy controls.

One of the goals of the study was to define the gut microbial perturbations in pediatric patients with newly diagnosed ITP compared to normal controls. This was accomplished by examining the initial gut microbial populations in pediatric patients with newly diagnosed ITP compared to age and gender matched healthy controls. It was thought that certain contents in the gut favoring inflammatory may be present in children with ITP. When analyzing the data collected there is a significant difference in diversity of the gut microbiome in pediatric patients with ITP compared to healthy controls as well as patients with acquired aplastic anemia (who did not have any changes compared to healthy controls). There was also a strong trend towards a gut microbiome that was less rich in pediatric patients with ITP compared to the two other cohorts. Microbiomes that are less diverse and less rich are usually indicative of a microbiome imbalance which points to underlying illness and/or inflammation.

These data are particularly exciting as they show for the first time that pediatric patients with ITP have an imbalanced gut microbiome.

Another goal of the study was to characterize the autoimmune environment present at diagnosis in patients with ITP. An analysis of each individual bacterial species showed significant overrepresentation of *E. coli* and *B. fragilis* in pediatric patients with ITP compared to other groups. This was done using the laboratory tests, and specialized immunology tests (such as flow cytometry and ELISA), and investigating

specific immune biomarkers present at diagnosis in pediatric patients with primary ITP. The particular patterns of overrepresentation and underrepresentation of certain microbiome species is further indication of an underlying inflammatory and/or ill state.

An analysis was conducted on metabolic pathways that impacted by the gut microbiome in pediatric patients with ITP. There was a decrease in various biological components (specifically, Aminoacyl-tRNA biosynthesis responsible for part of protein synthesis, Ribosome pathways; the site of protein synthesis, Homologous recombination, RNA degradation, Nucleotide excision repair and mismatch repair). This means that pediatric patients with ITP have less-active metabolic pathways which help to both build up and repair proteins.

Patients with ITP had overrepresentation of *E. coli* and *B. fragilis* while they had a decrease in *A. finegoldii* and *F. prausnitzii* dysregulation caused by a gut microbial imbalance. Furthermore, biochemical pathways analysis revealed an underrepresentation of small chain fatty acids and particularly butyrate (responsible for breaking down fiber) production pathways in the pediatric ITP cohort compared to the other two cohorts. This is of particular importance given the recent demonstration that butyrate is protective against inflammatory and immune-mediated diseases such as chronic graft versus host disease, and post-allogeneic hematopoietic stem cell transplant (Markey KA et al Blood 2020).

Lastly, the third goal of the study was to develop a pilot predictive-modeling algorithm based on gut microbiome imbalances and inflammation at diagnosis to identify patients at risk for chronic refractory disease. Using data obtained from Aims 1 & 2 and combining it with clinical data extracted from the electronic medical record (EMR), a score was developed to predict development of chronic or hard-to-treat ITP.

Overall, data obtained from this pilot project thus far shows for the first time that pediatric patients with ITP have a dysregulated and imbalanced gut microbiome compared to healthy controls. Given the complex interactions between the gut microbiome and the host immune system, understanding the gut microbiome and its contribution to the pathogenesis and mechanism of ITP is of critical importance. The pathways analysis showed decreased production of butyrate which could theoretically cause a primed host milieu to enable inflammatory and autoimmune conditions.

This project is on-going, and investigators are currently working on 3 goals simultaneously: 1) expand the ITP group and enable sequencing of additional patients with an additional 10 samples from patients; 2) use validated flow cytometry to understand the antibody coating of gut microbes in patients with ITP and if/how the gut microbe imbalance or ITP itself has implications for antibody coating; and 3) metabolic analysis to measure levels of short chain fatty acids. It is expected that the data will provide a basis for understanding the potential contribution of the gut microbiome to the pathophysiology of ITP.

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PDSA has designed our research program to specifically focus on patient priorities and funds studies that will make a significant impact on ITP diagnosis, therapies, and quality of life. If you'd like to donate to our research fund, please visit <https://www.pdsa.org/pdsa-donation.html>.