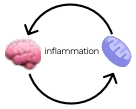
Disease: **Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)**

ME/CFS Hypotheses



Vicious feedback loops in various pathways perpetuate chronic inflammation. **Risk factors** may be gene mutations. **Triggers** may be pathogenic infections.

The Neuroinflammation Hypothesis: a feedback loop of inflammation in nerve cells (1).

The Metabolic Trap Hypothesis: a feedback loop of inflammation in mitochondria, the energy factories of all cells (2).

Study Overview

Take **antibiotics** to escape the **metabolic trap** & induce remission events.

Collect samples during **remission events**.

Analyze **gene expression data** for measuring changes in immune cells that may drive **neuroinflammation**.

Study Procedure*

2/2/23-3/19/23
Prep phase
(Prebiotic/Postbiotic + Probiotic)

3/20/23-4/2/23
Acute phase
(Prebiotic/Probiotic + Probiotic + AmoxiClav antibiotics + supplements)

4/3/23-7/1/23
Recovery phase
(Probiotic + supplements)

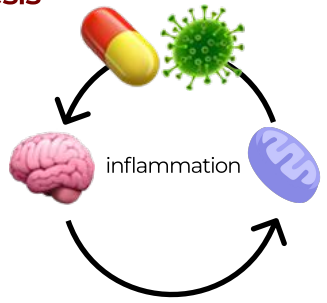
Patient scientist

Dr. Tess Falor



RemissionBiome Study Hypothesis

Disrupt feedback loops of chronic, systemic inflammation via the microbiome.



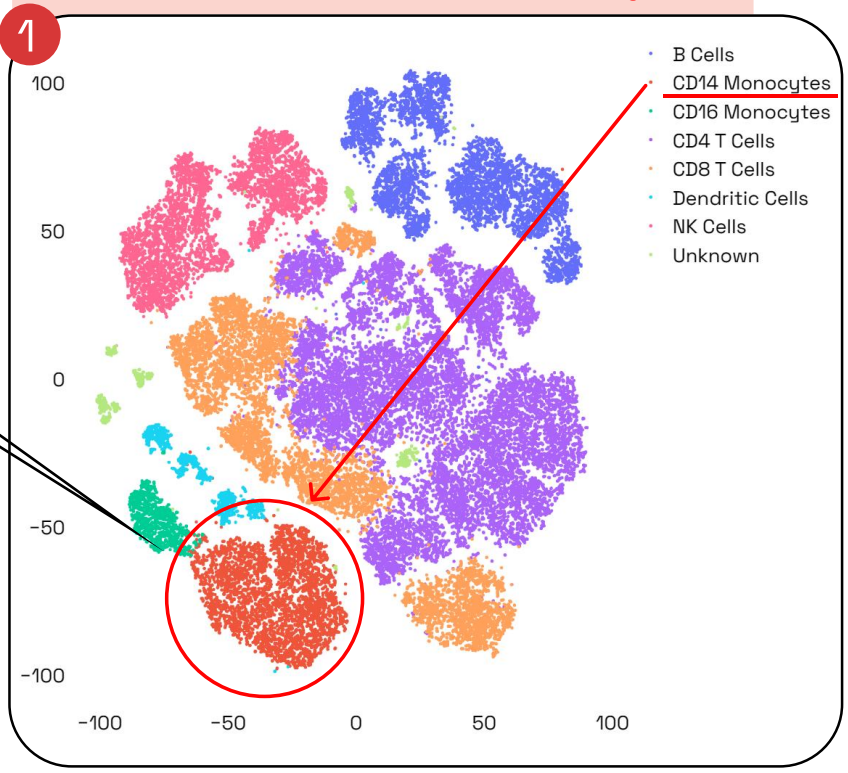
	1	2	3	4	5	6	7	8	9
Date:	1/30/23	2/8/23	3/2/23	3/21/23	3/24/23	3/28/23	4/5/23	4/10/23	5/22/23
Sample:	Baseline 1	Baseline 2	Baseline 3	Event 1	Event 2	Event 3	New Baseline 1	New Baseline 1	New Baseline 1
Antibiotics:				Day 2	Day 5	Day 9			

Sources:
(1) Neuroinflammation, https://me-pedia.org/wiki/Neuroinflammation#cite_note-:3-8
(2) Metabolic Trap, https://me-pedia.org/wiki/Metabolic_trap

**Do not replicate this procedure without consulting your medical provider. Review additional disclaimers on page 6.*

! Cell Type Flagged: CD14 Monocytes

1

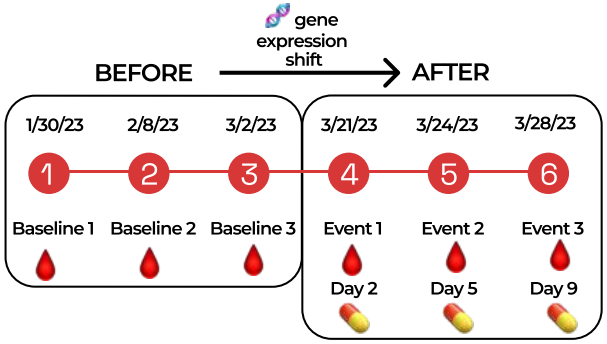


📚 **Supporting Findings:**

"monocytes exhibited the strongest signals of dysregulation [in ME/CFS immune systems]" (Grimson, et al. 2024)

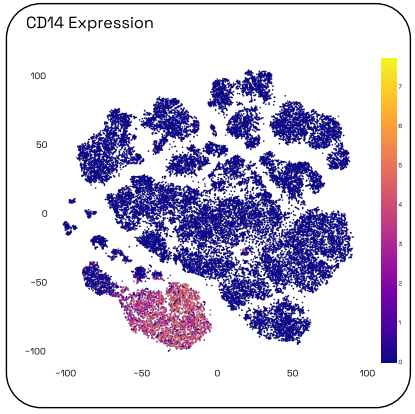
✅ This cell type is typically found in relatively low abundance! (It's rare to detect as many as we did!) We're more confident that our gene "hits" will be *specific* signals that are *unique* to these rare cells.

We flagged a specific type of immune cell. Continue to the next page & see how these cells changed over time 🧬...



How did we flag this cell population?

Machine learning (ML) models trained on ImYoo's database!



We tell the model which cells are known to express specific genes.

🏝️ The model clusters all the cells in our sample. Each island is a distinct type of cell.

Left: We identify a cluster of CD14 monocytes.

We used artificial intelligence (AI) to generate this plot, which is called a "t-SNE" ("tiz-nee").

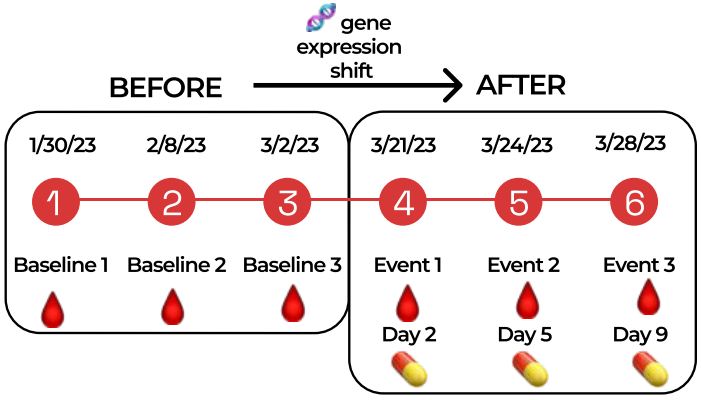
N-1 Case Study: Cellular gene expression post intervention in chronic fatigue syndrome

2 Number of gene "hits": 29

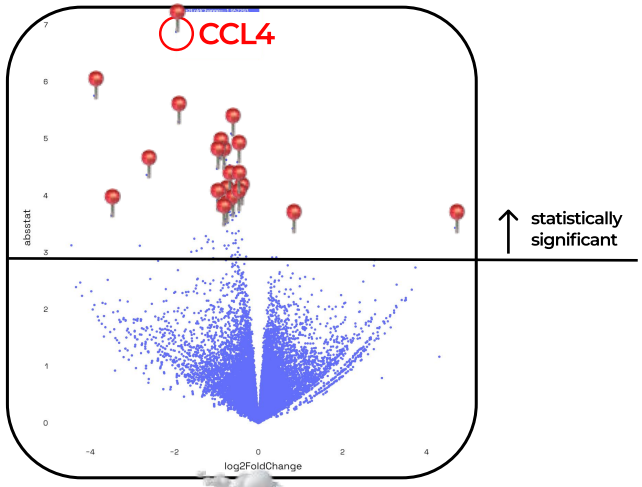
	cell_type	participant_id	num_genes	signal_strength
0	CD14 Monocytes	P363	29	151.372205
1	CD4 T Cells	P363	0	0.799869
2	CD8 T Cells	P363	0	3.539320
4	NK Cells	P363	0	0.103601
5	CD16 Monocytes	P363	0	0.024183
6	B Cells	P363	0	0.290662
7	Dendritic Cells	P363	0	4.798657

Signal Strength: High

✅ Unique to a cell type
✅ Unique to a rare cell type
✅ Many of the top "hits" are known immune modulators
🧬 29 genes *shifted* in gene expression before vs during the remission event



3 Gene of interest: CCL4



📚 Supporting Findings:

"Specifically, we observed upregulation of CCL4... [after exercise in ME/CFS patients]" (Grimson, et al. 2024)

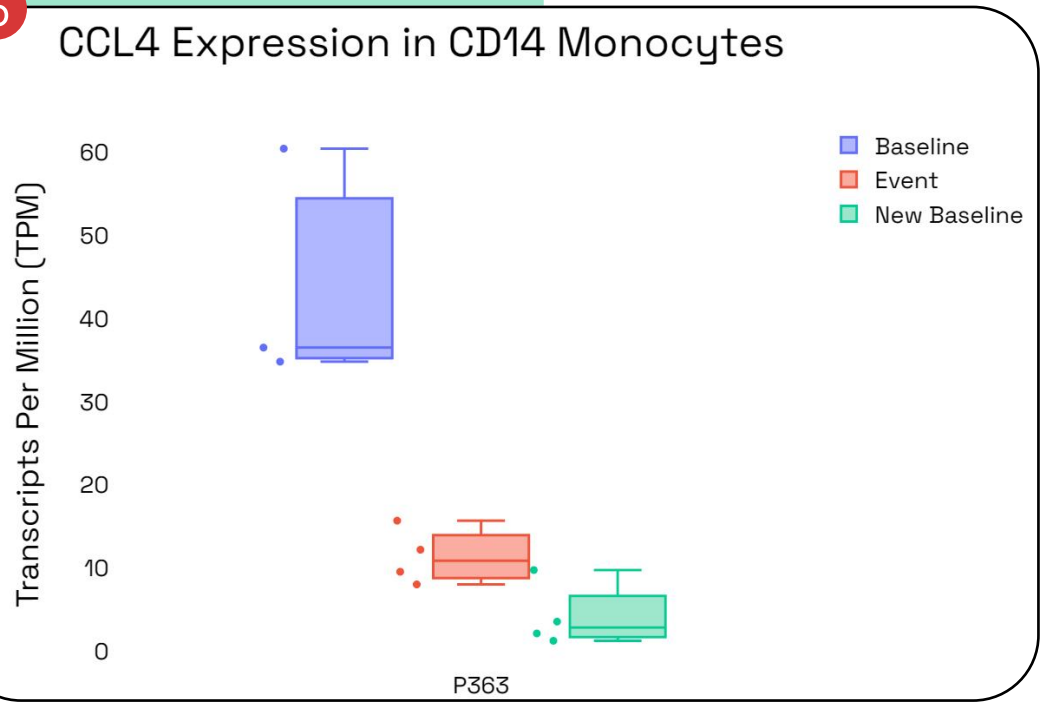


This is a volcano plot! Look above the horizontal line for statistically significant gene shifts.

Learn to interpret our plots for yourself! Visit our YouTube channel for explainers. 👉

Novel Insight

5



👉💡 Gene CCL4 is downregulated during ME/CFS remission events and *continues to decrease* in gene expression for months following antibiotic intervention.

📚 **Supporting Findings:**

"Specifically, we observed upregulation of CCL4 [after exercise in ME/CFS patients]*..." (Grimson, et al. 2024)

*exercise can exacerbate ME/CFS symtpoms, like post-exertional malaise

🤔 This is an N of 1 study. Let's think critically about these findings:

This study observed the changes in 1 person's immune system. In addition to the antibiotic intervention, there are numerous alternative explanations for these results.

What we *can* say is that self-organized, self-administered intervention studies by N of 1 patient scientists can build hypotheses to follow-on study.

Alternative Explanations: Ask, "What else could've caused what I am seeing?"

🤔 Consider **disease variability:**
E.g. The patient has a unique manifestation of ME/CFS that is caused by bacterial-induced immune activity.

🤔 Consider **confounding variables:**
E.g. This study took place over seasonal changes, so the patient might have had immune activation from seasonal allergies.

🤔 Consider **technical artifacts:**
E.g. Samples were batched separately for processing by a person in the lab. Different batches are different conditions to consider.

🤔 Consider **human error:**
E.g. The patient might've packaged their samples better over time.

N-1 Case Study: Cellular gene expression post intervention in chronic fatigue syndrome

Comments from the Experts



"This is actually really interesting because it suggests that **the microbiome** (or microbial metabolites) can impact immune cell activation in this ME/CFS patient."
Dr. Lavanya Visvabharathy, immunologist @Northwestern University

Recall the "metabolic trap hypothesis"!

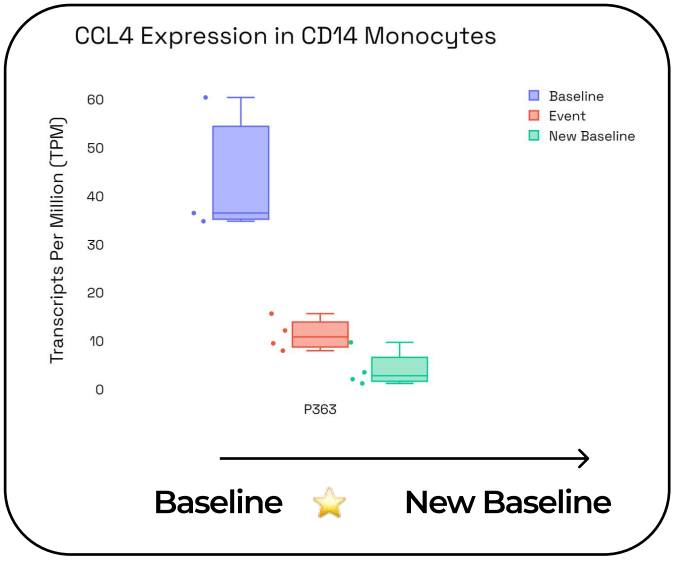


"A lot of people [with ME/CFS] **can't leave their house** or they're even bed bound... They can't take part in these research studies where you have to actually travel and go into the clinic."
Dr. Tess Falor, patient scientist @RemissionBiome

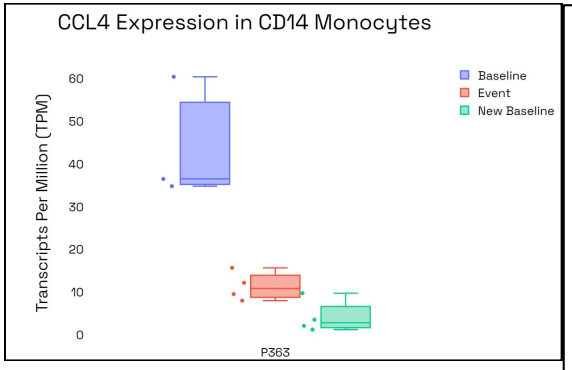
Patient-centric sampling makes the impossible possible!



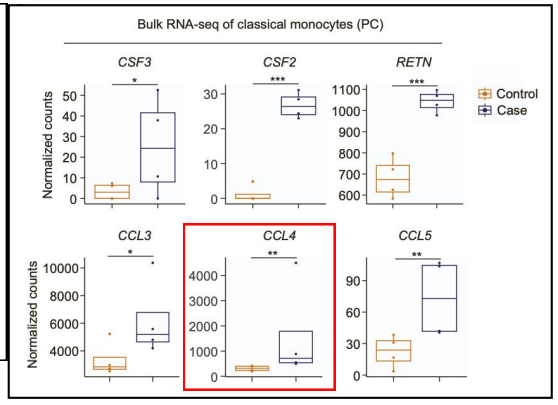
"Can you modulate metabolism through the microbiome?"- ImYoo
"Absolutely."
Dr. Rob Phair @Integrative Bioinformatics



ImYoo CCL4 findings with single-cell RNA sequencing:



Grimson CCL4 findings with bulk RNA sequencing:





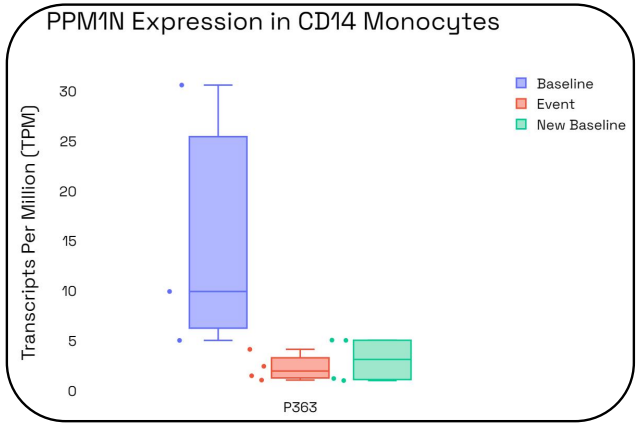
"This is a great study... it really sets up a strong motivation to extend this kind of work beyond an N of 1."
Dr. Andrew Grimson @Cornell University

(Left) ImYoo's report from scRNA-seq shows **decreased CCL4** in CD14 monocytes during **reduced ME/CFS symptoms.** (Right) Figure 6c from Grimson's supplementary info. Grimson uses bulk RNA sequencing to show **increased CCL4 activity** in immune cells during **elevated ME/CFS symptoms.** Both ImYoo and Grimson point to CCL4 gene activity as correlating with ME/CFS symptoms. (Grimson et al.)

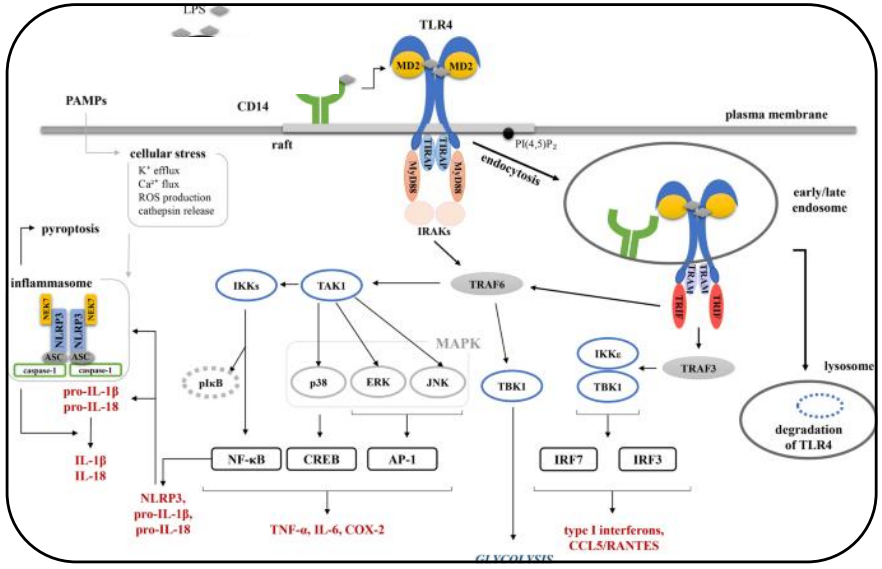
Quotes from Dr. Rob Phair & Dr. Andrew Grimson can be found in the RemissionBiome Roundtable discussion at timestamps 39:40 & 24:48, respectively.

Supplement: Relating a gene "hit" to a molecular pathway

Gene "hit": PPM1N is downregulated



TLR4 Pathway in CD14 Monocytes



Above: PPM1N is a gene encoding a protein phosphatase in the PPM family. This family of phosphatases play a role in toll-like receptor (TLR) signaling. TLR4 is a transmembrane receptor that plays a key role in detecting and combating bacterial infections. Some bacteria produce a protein called lipopolysaccharides (LPS). LPS binds to TLR4, triggering a molecular pathway that mounts the immune system's defenses. Monocytes specifically are upregulated in the presence of LPS-producing bacteria. These bacteria are associated with not just chronic fatigue syndrome, but also play a role in also Lyme disease and other chronic inflammatory conditions. Figure provided by Ciesielska, et al.

Disclaimers:

***Medical Disclaimer:** Both ImYoo and RemissionBiome would like to emphasize that this N of 1 study is not intended to be replicated without proper medical supervision. For that reason, this article does not disclose the details of Falor's antibiotic regimen. This N of 1 case study is not intended to offer any clinical or scientific advice, nor conclusions, about diseases such as ME/CFS, Long COVID, or any other conditions cited in these writings.

***Scientific Disclaimer:** ImYoo did not inform the design of this study intervention. Rather, ImYoo's role was solely to provide observational data and analysis. The observations were made in one individual (i.e. an N of 1 study). Therefore, causality cannot be confirmed from ImYoo's findings. ImYoo cannot confirm that the observed signals were due to the reported remission event, rather than a typical reaction to antibiotics. To distinguish between disease-specific response and general antibiotic response, ImYoo would suggest an additional study that measures immune response to antibiotic treatment. ImYoo acknowledges that, aside from the RemissionBiome intervention, other factors could have contributed to the results presented in this article. For more resources on how to interpret N of 1 studies, consult this article's supplementary ImYoo report.

Links

- ImYoo Raw Data and Gene Tables
- RemissionBiome website
- ImYoo website
- RemissionBiome Rountable Discussion (YouTube video)

Publications cited in this Report:

Ciesielska, A., Matyjek, M. & Kwiatkowska, K. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cell. Mol. Life Sci. 78, 1233–1261 (2021). https://doi.org/10.1007/s00018-020-03656-y

Grimson, et al. *Single-cell transcriptomics of the immune system in ME/CFS at baseline and following symptom provocation,* 2024

VanElzakker MB, Brumfield SA, Lara Mejia PS. Neuroinflammation and Cytokines in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): A Critical Review of Research Methods. Front Neurol. 2019 Jan 10;9:1033. doi: 10.3389/fneur.2018.01033. Erratum in: Front Neurol. 2019 Apr 02;10:316. Erratum in: Front Neurol. 2020 Sep 17;11:863. PMID: 30687207; PMCID: PMC6335565.