Long Covid Report

Intro:

Biomarker Results:

- **Immune Profile:**
  - Elevated IL-4 and RANTES (CCL5) with low IL-13, low TNF-α, and low GM-CSF suggest a Th2-skewed, antibody-promoting environment with chronic immune activation.
  - Normal CD3 and CD4 counts but low CD8+ T cells point toward possible T cell exhaustion.
  - Elevated IgG4 (151.8 mg/dL) with negative standard autoantibodies indicates a non-inflammatory, functional autoantibody process—consistent with GPCR autoantibody dysfunction.
- **CellTrend GPCR Auto-Antibodies**:

Receptor	Titer (U/mL)	Interpretation
β-1-adrenergic	81.4	**Positive**
β-2-adrenergic	59.5	**Positive**
α-1-adrenergic	56.7	**Positive**
AT1R	50.9	**Positive**
ETAR	50.0	**Positive**
MAS1	75.1	**Positive**
ACE-2	15.0	Positive
M3 (muscarinic)	46.0	**Positive**
M4 (muscarinic)	51.6	**Positive**
M5 (muscarinic)	12.6	At-risk
CXCR3	16.7	Positive
Stab1	11.8	Positive
FGFR-3	14.0	At-risk
TSH-DS IgM	11.3	Positive

- *Pattern:* multi-target **GPCR/RAS antibody swarm** dominated by IgG4 → mechanistically fits **dysautonomia (HR/BP swings), brain-stem autonomic "hits," and GI burning**.
- **Viral Reservoir Assessment**
  - **S1 Immune Subset Panel:** 0 % S1 protein in monocytes; all monocyte subsets low → persistent spike antigen unlikely.
- **Theory**
  Primary driver: broad **GPCR auto-antibody dysfunction** (IgG4-skewed).
  Amplifiers: intermittent mitochondrial crash, endothelial reactivity ± microclots, and CD8-T exhaustion.
  Low probability of classic T-cell–mediated or innate autoinflammatory disease.

Next Steps & Monitoring Plan

1. **Target the Auto-Abs**

- **Schedule:** therapeutic plasma-exchange (TPE)
- **Stabilize rebound:**
    1. *Cycle 1* → **Efgartigimod** 10 mg/kg IV weekly×4 beginning 2 weeks post-TPE.
    2. *Cycle 2* → repeat on flare or IgG4/GPCR AAb rise (≥ 60 days wash-out).
- **Escalation gate:** if GPCR titers or plasmablasts surge again → single course **Obinutuzumab 1 g IV day 0/14** (type-II CD20 depleter).

2. **Peptide & Small-Molecule Core**
    - **Tier 1:** Thymosin α-1 1.5 mg SC BIW, **SS-31 10 mg SC Mon–Fri** (8 w on / 4 w off).
    - **Tier 2 adjuncts:** BPC-157 300 µg SC 5 d/wk, NAD^+ 250 mg IV q-week × 8, Epitalon 10 mg SC q-HS × 10 d.
    - **Medications:** continue **LDN 4.5 mg HS**, **Melatonin 3–5 mg HS**; **Rapamycin 3 mg PO q-4 wks** only if CD8 exhaustion persists.
3. **Supportive / Lifestyle**
    - **Mito-stack:** CoQ10 200 mg, Acetyl-L-Carnitine 1 g, Alpha-lipoic acid 300 mg AM.(+ methylation support)
    - **Endothelial & microclot guard:** Atorvastatin 10–20 mg HS + low-dose aspirin 81 mg HS pending microclot panel.
    - **GI burning:** Pantoprazole 40 mg AM, Sucralfate 1 g QID
4. **Lab & Imaging Schedule:**

Time-point	Key tests
Baseline	GPCR panel, IgG4, CBC/CMP, CD8 flow (+exhaustion markers), lactate/pyruvate, F2-isoprostanes, microclot panel (fibrinaloid), HRV 24-h
Week 4 (post-TPE)	GPCR, IgG4, IgG, cytokines, CMP
Week 8 (efgartigimod nadir)	Same + plasmablast % (CD27++/CD38++)
Week 16	Repeat cytokines, CD8 function, microclot panel
Quarterly	GPCR + IgG4, NK/T exhaustion, mito markers, autonomic symptom score

- **Expected Biomarker Trajectory**
    - **IgG4 & GPCR AAbs:** ↓ 50-70 % by week 8; plateau with efgartigimod maintenance.
    - **IL-4, RANTES:** target ≥30 % decline; **IL-13** may rise toward normal.
    - **CD8 counts / PD-1↓:** gradual recovery over 3–6 mo if Tα-1 effective.
    - **Mito markers (lactate ↑, ATP ↓):** improved post-SS-31/HBOT block.

> *BLUF:* The new CellTrend panel confirms a broad, IgG4-weighted GPCR auto-antibody storm that maps to your dysautonomia, gastric burning, and exertion-triggered crashes. The updated plan prioritises **(1) rapid removal (TPE), (2) FcRn blockade (efgartigimod) to prevent rebound, and (3) deeper B-cell reset (obinutuzumab) only if needed**, layered with mitochondrial, endothelial, and autonomic supports.

Integrated Framework for Long COVID Mechanisms

Long COVID appears to be driven by a cascade of interrelated immune dysfunctions. The model divides the underlying immune dysregulation into four overarching “buckets” with secondary and tertiary pathways that contribute to downstream effects such as microclotting, hypometabolism, mitochondrial dysfunction, and neuropathies.

The Four Overarching Buckets

1. **Immune Suppression**
   - **Foundation:**
     - Represents reduced or exhausted immune cell function (e.g., low CD8+ T cells, reduced NK cell activity).
     - May result from chronic antigen stimulation and can impair pathogen clearance.
   - **Downstream Effects:**
     - Increased susceptibility to infections, persistent viral fragments, and chronic immune activation that indirectly drives other dysregulations. Reduced pathogen clearance, chronic infections, and persistent antigen presence, which further stimulate dysregulation.
   - **Testing** :
     - Flow cytometry for T cell and NK cell function, proliferation assays, and cytotoxicity tests.
       - **Flow Cytometry for T Cell Exhaustion Determines:
         1. T Cell Activation & Exhaustion:
            - **PD-1 (Programmed Death-1):** High expression suggests exhaustion.
            - **CTLA-4:** Another inhibitory receptor seen in exhausted T cells.
            - **LAG-3, TIM-3, TIGIT:** Additional exhaustion markers (often co-expressed with PD-1).
            - **CD38, HLA-DR:** Indicators of chronic activation (can precede exhaustion).
            - **CD57:** Associated with terminal differentiation/exhaustion in CD8+ T cells.
            - **Loss of CD28/CD127 (IL-7R):** Common in exhausted or senescent T cells.
         2. **T Cell Subset Differentiation:**
            - **Naïve (CD45RA+/CD62L+)**
            - **Effector Memory (CD45RO+/CD62L−)**
            - **Central Memory (CD45RO+/CD62L+)**
            - **Exhausted/Senescent (CD57+/CD28−/CD127−)**
         3. **Proliferation & Functional Capacity:**
            - **Ki-67:** Proliferation marker (low in exhausted cells).
            - **Intracellular IFN-γ, TNF-α, IL-2 production:** Exhausted T cells often lose the ability to produce these cytokines effectively.
       - **Flow Cytometry for NK Cell Function**
         1. NK Cell Subsets (CD3− CD56+ Cells)
         - CD56dim/CD16+ (Cytotoxic NK cells)**: Main killers.**
         - CD56bright/CD16− (Regulatory NK cells)**: More cytokine production, less cytotoxicity.**
         2. Activation & Exhaustion Markers
         - NKG2A vs. NKG2C Ratio:
         - NKG2A-high: **Suggests suppression/exhaustion.**
         - NKG2C-high: **Suggests hyperactivation (common in viral reactivation).**
         - CD57+ Expression: **Marker of terminal differentiation (exhausted NK cells).**
         - PD-1, LAG-3, TIM-3: **Can also be seen in NK exhaustion.**
         - CD107a (LAMP-1): **Marker of degranulation/cytotoxicity.**
         - Perforin & Granzyme B Production:** Indicates cytotoxic function.
       - **B-cell flow cytometry panel**,
         - CD19 and CD20 — total B-cell count
         - CD27 — memory B-cells
         - CD38 — plasmablasts / actively antibody-secreting cells
         - (optional)
         - IgD — to distinguish naive vs. switched memory B-cells
         - CD138 — plasma cells (sometimes included)

2. **Autoimmune Antibody (B Cell–Driven) Dysfunction**
   - **Foundation:**
     - Involves overproduction of autoantibodies—often against receptors like β-adrenergic, muscarinic, ACE2, AT1R, and ETAR.
     - In long COVID, a Th2-skewed environment (elevated IL-4, high RANTES) may favor production of G-protein–coupled receptors [GPCRs] autoantibodies, often in the IgG4 subclass, which cause "functional" receptor disruption without heavy inflammation.
   - **Downstream Effects:**
     - Receptor dysfunction leads to autonomic instability (e.g., tachycardia, blood pressure swings), gastrointestinal dysregulation, and vascular issues.
   - **Evidence:**
     - Elevated IL-4 and IgG4, high CCL5 (RANTES).
   - **Advanced Testing:**
     - Specialized GPCR autoantibody panels (e.g., CellTrend), detailed immunoglobulin subclass analysis, and B-cell subset assessments.
3. **Autoimmune T Cell–Driven Dysfunction**
   - **Foundation:**
     - Involves dysregulated T cell responses—either overactivation (autoreactivity) or exhaustion.
     - Chronic stimulation can lead to high IFN-γ production and, if severe, may show altered T cell activation/exhaustion markers (PD-1, CTLA-4, CD38, HLA-DR).
   - **Downstream Effects:**
     - May contribute to neuroinflammation, cognitive dysfunction, and autonomic instability.
     - However, in many long COVID cases, overall T cell counts (CD3, CD4) are normal while CD8+ T cells are low, suggesting exhaustion rather than aggressive autoimmunity.
   - **Evidence:**
     - Elevated IFN-γ, normal CD3 and CD4 counts, but low CD8+ T cells.
   - **Advanced Testing:**
     - Functional T cell assays (proliferation, cytokine production), detailed phenotyping (flow cytometry panels for Tregs, memory, and regulatory T cell subsets), and TCR clonality studies (research-level).
4. **Autoinflammatory Processes**
   - **Foundation:**
     - Characterized by innate immune system overactivity without a prominent adaptive (autoantibody or T cell) signature.
     - Typically presents with episodic fevers and elevated acute-phase reactants (e.g., IL-1β, IL-18, SAA), though in many long COVID patients, IL-6 and TNF-α may be normal or low.
   - **Downstream Effects:**
     - Can drive systemic inflammation that indirectly affects other systems, such as endothelial function and mitochondrial metabolism, and possible microvascular damage.
   - **Advanced Testing:**
     - Expanded cytokine panels (including IL-1β, IL-18, IL-1RA), high-sensitivity CRP, and genetic panels in suspected hereditary autoinflammatory syndromes.

Downstream Pathways and Testing Considerations

These primary dysregulations often lead to further issues:

- **Mitochondrial Dysfunction:**
  - Reduced ATP production, increased oxidative stress, and impaired energy availability contribute to post-exertional malaise (PEM) and autonomic dysregulation.
- **Endothelial Dysfunction & Microclotting:**
  - Dysregulated endothelial cells and microclot formation can impair blood flow, causing tissue hypoxia and exacerbating autonomic instability.
- **Neuroinflammation & Gut Dysbiosis:**
  - Inflammatory processes may affect the brain (e.g., brain fog, cognitive dysfunction) and gut (e.g., leaky gut, GI dysmotility), further fueling systemic immune dysregulation.

3.1 Mitochondrial Function Tests

- **Lactate/Pyruvate Ratio (Rest & Post-Exertion)**
  - Helps confirm if the patient is shifting into **anaerobic metabolism** too quickly, which aligns with **post-exertional malaise**.
- **CoQ10 Blood Levels, Oxidative Stress Markers (F2-Isoprostanes)**
  - Evaluate **oxidative damage** and adequacy of the electron transport chain.
- **Acylcarnitine Profile**
  - Screens for **fatty acid oxidation defects** that could exacerbate fatigue.
- **Organic Acids Test (OAT)**
  - Checks for **Krebs cycle metabolites** and can reveal blocks in energy production.

Why It Matters: Chronic immune activation and autoimmunity frequently impair mitochondrial function, leading to low energy states and high oxidative stress. Addressing these issues (e.g., with SS-31, NAD+, or CoQ10) can significantly improve stamina and reduce PEM.

3.2 Autonomic/ANS Tests

- **Tilt-Table Test or Heart Rate Variability (HRV)**
  - Evaluates **dysautonomia**, such as orthostatic intolerance or POTS, which often accompanies GPCR autoantibody dysfunction (β-adrenergic, muscarinic).
- **24-Hour Holter Monitor**
  - Tracks **inappropriate heart rate spikes** or arrhythmic episodes often seen with autoimmunity to β2-adrenergic receptors.

  Why It Matters: Dysautonomia is a hallmark of GPCR autoantibody-mediated syndromes (e.g., POTS). Objective confirmation supports interventions like **beta-blockers**, **ARBs**, or **IVIG**.

3.3 Immune Profiling

- **Expanded Cytokine Panels** (Pro- & Anti-Inflammatory)
  - Confirms the **Th2 skew** (IL-4, IL-13) or reveals hidden Th1/Th17 components (IL-17, IL-21).
- **GPCR Autoantibody Panels (CellTrend)**
  - Specifically checks for **anti-β2-adrenergic**, **anti-muscarinic**, **anti-ACE2**, and others.

- **B-cell Subset Analysis**
  - Clarifies whether particular subsets (memory B cells, plasmablasts) are overactive.
  Why It Matters: Definitive proof of **ACE2 or β2 GPCR autoantibodies** can change clinical management (e.g., more aggressive immunotherapies like IVIG/plasmapheresis).

3.4 Microclots & Hypercoagulability

- **Fibrin Amyloid Microclot Testing**
  - Research-grade test (specialized labs) to detect **microclots** not seen on routine assays.
- **D-Dimer**
  - Common measure of fibrinolysis; however, it may **miss microclots** if not overtly elevated.
- **TEG/ROTEM**
  - **Real-time clotting analyses** to see if there's a hypercoagulable trend.
- **VWF & ADAMTS13 Imbalance**
  - **Endothelial dysfunction** marker, potential pro-thrombotic state.
  Why It Matters: Microclots can exacerbate **tissue hypoxia**, perpetuate **fatigue**, and even worsen **dysautonomia**. When present, therapies aimed at improving **microvascular flow** (e.g., antiplatelet agents, nattokinase, or more advanced interventions) may be warranted.

3.5 Endothelial Dysfunction Assessments

- **ICAM-1, VCAM-1, E-Selectin**
  - **Endothelial activation markers** indicating chronic vascular inflammation.
- **EndoPAT or Flow-Mediated Dilation (FMD)**
  - Non-invasive tests for **microvascular reactivity** and nitric oxide-mediated dilation.
  Why It Matters: Endothelial dysfunction is a downstream manifestation of **immune dysregulation**, microclots, and possibly direct ACE2 disruption. Normalizing endothelial function can improve symptom severity, especially in **exercise intolerance**.

Test Results and Laboratory Findings

The following laboratory results were discussed as part of evaluation:

A. Cytokine and Immune Marker Panel

- **IL-2:** 3.1 pg/mL (Normal)
- **IL-4:** 8.1 pg/mL (High)
  • Supports a Th2-skewed response (promotes antibody production).
- **IL-6:** 2.1 pg/mL (Normal)
- **IL-8:** 11.3 pg/mL (Normal)
- **IL-10:** 1.1 pg/mL (Normal)
- **IL-13:** 0.8 pg/mL (Low)
- **GM-CSF:** 3.6 pg/mL (Low)
- **SCD40L:** 2171.8 pg/mL (Normal)

- **CCL3 (MIP-1α):** 2.2 pg/mL (Low)
- **CCL4 (MIP-1β):** 11.3 pg/mL (Normal)
- **CCL5 (RANTES):** 12,780.2 pg/mL (High)
  • Indicative of chronic immune cell recruitment.
- **TNF-α:** 2.4 pg/mL (Low)
- **IFN-γ:** 8.9 pg/mL (High)
  • Suggests chronic T cell/NK cell activation.
- **VEGF:** 10.9 pg/mL (Normal)
- **Long Hauler Index:** 1.06 (High; normal < 0.70)

B. GPCR Autoantibody Panel

- **anti-Muscarinic Cholinergic Receptor-5 Antibodies:** 12.6 U/mL (At Risk)
- **anti-FGF Receptor-3 Antibodies:** 14.0 U/mL (At Risk)
- **anti-Muscarinic Cholinergic Receptor-3 Antibodies:** 46.0 U/mL (Positive)
- **anti-Muscarinic Cholinergic Receptor-4 Antibodies:** 51.6 U/mL (Positive)
- **anti β-1-adrenergic Receptor Antibodies:** 81.4 U/mL (Positive)
- **anti β-2-adrenergic Receptor Antibodies:** 59.5 U/mL (Positive)
- **anti-Stab1 Antibodies:** 11.8 U/mL (Positive)
- **anti-ACE-2 Antibodies:** 15.0 U/mL (Positive)
- **anti-MAS1 Antibodies:** 75.1 U/mL (Positive)
- **anti-CXCR3 Antibodies:** 16.7 U/mL (Positive)
- **anti-TSHDS-IgM Antibodies:** 11.3 U/mL (Positive)
- **anti ETAR Antibodies:** 50.0 U/mL (Positive)
- **anti AT1R Antibodies:** 50.9 U/mL (Positive)
- **anti α-1-adrenergic Receptor Antibodies:** 56.7 U/mL (Positive)

C. Lymphocyte Subset Analysis

- **CD3 T Mature Cells:** 78.6% (Normal); Absolute: 2512 cells/μL (Normal)
- **CD4+ T Cells:** 45.27% (Normal); Absolute: 663 cells/μL (Normal)
- **CD8+ T Cells:** 32.31% (Normal %); Absolute: 166 cells/μL (Low)
  • Low CD8+ count may indicate T cell exhaustion or chronic activation.

D. Standard Bloodwork

- **CBC/Diff:**
  • WBC, RBC, Hemoglobin, Hematocrit within normal limits.
  • RDW slightly low; Platelets within range.
- **Autoimmune Markers:**
  • SSA, SSB, Sm, Scl-70, RNP, Centromere, Chromatin, ssDNA, dsDNA, ANA screens are all normal.
- **Immunoglobulin Subclasses:**
  • Notably, IgG4 is elevated at 151.8 mg/dL (reference 4–86 mg/dL).
- **Metabolic and Thyroid Panels:**
  • TSH (2.11 uIU/mL) and T4, T3 are normal.
- **Infection Markers:**
  • Herpesvirus PCR tests negative; EBV IgG is elevated (indicative of past exposure), IgM negative. • Lyme disease Western blot negative.

E. S1 Immune Subset Panel

- **Monocyte Subset Percentages:**
  - **Non-classical (CD14^low/CD16+):** 1.51% (Low; reference 25.50–40.00)
  - **Intermediate (CD14+/CD16+):** 3.15% (Low; reference 4.60–13.40)
  - **Classical (CD14++/CD16–):** 11.86% (Low; reference 34.40–51.20)
- **S1 Protein Detection:**
  - 0.0% in all monocyte subsets
    • Indicates no detectable persistent SARS-CoV-2 S1 protein in monocytes.

F. Additional Immune Phenotyping & T Cell Function

- **Senescent CD8+ T Cells:**
  - Further analysis (via flow cytometry for CD57, KLRG1, PD-1, and loss of CD28) is warranted to assess T cell exhaustion.
- **General Immunophenotyping:**
  - Detailed profiling of T cell subsets (Th1, Th2, Tregs, memory cells) and NK cell markers would further elucidate immune dysregulation.

note

overall systemic inflammation is within normal limits:
• CRP (<0.5 mg/dL) – Well within the normal range (0.0–1.0 mg/dL), suggesting no significant acute inflammatory response.
• ESR (3 mm/hr) – Low and within the normal range (<15 mm/hr), which further supports the absence of systemic inflammation.
• C3 (108 mg/dL) and C4 (21 mg/dL) – Both within normal limits, which does not suggest an active inflammatory process.
• Autoimmune markers (SSA, SSB, Sm, Scl70, RNP, Centromere, Chromatin, ssDNA, dsDNA, ANA screens) – All normal, indicating no apparent autoimmune inflammation.
• CBC and metabolic panel – No significant abnormalities that would suggest an inflammatory condition.
• IgG4 is elevated, but in isolation, it may not necessarily indicate active inflammation.

Matching Test Results to the Four Buckets

1. **Immune Suppression**
   - **Evidence:** Low absolute CD8+ T cells; possible markers of T cell exhaustion.
   - **Testing Needed:** Advanced T cell functional assays (exhaustion markers such as PD-1, CTLA-4) and NK cell functional studies.
2. **Autoimmune Antibody Dysfunction**
   - **Evidence:**
     - Elevated IL-4 and very high CCL5 (RANTES) suggest a Th2-skewed environment.
     - Elevated IgG4 without other classical autoimmune markers.
   - **Testing Needed:** Specialized GPCR autoantibody panels (e.g., CellTrend) and detailed B cell subset analysis.
   - **Clinical Implication:** Points toward a functional autoantibody (possibly IgG4-mediated) process affecting receptor signaling, leading to dysautonomia and other symptoms.
3. **Autoimmune T Cell Dysfunction**
   - **Evidence:**
     - Elevated IFN-γ may indicate chronic T cell activation, though overall T cell counts (CD3, CD4) are normal.

    - Low TNF-α and GM-CSF suggest lack of robust pro-inflammatory T cell activity, which is more consistent with exhaustion than a classic T cell autoimmune attack.
  - **Testing Needed:** Expanded T cell phenotyping (memory, activation, Treg function, proliferation assays).
4. **Autoinflammatory Processes**
  - **Evidence:**
    - Cytokine profile does not show markedly elevated IL-6 or TNF-α that would support a robust autoinflammatory state.
  - **Testing Needed:** Further cytokine profiling (e.g., IL-1β, IL-18, SAA) if episodic inflammatory symptoms occur.

Final Key Takeaways

- **Functional (IgG4) autoantibodies** against GPCRs/ACE2 drive many long COVID symptoms without overt inflammatory signals.
- **Downstream processes** (mitochondrial dysfunction, endothelial stress, and microclots) can **amplify or sustain** autoantibody production.
- **Advanced testing** for chronic infections, toxins, mast cell issues, and hormone imbalances is crucial before **plasmapheresis**, so you do not remove antibodies only to have them return quickly.
- A **phased, comprehensive approach**—immunomodulation + receptor stabilization + addressing underlying triggers—is typically the most effective way to break the vicious cycle of functional autoimmunity in long COVID.

Auto Antibodies (GPCP)

1. Introduction

Long COVID (Post-Acute Sequelae of SARS-CoV-2) is a multifactorial disorder involving:

- **Chronic or recurring immune activation**
- **Autonomic dysfunction** (often with dysautonomia, POTS-like symptoms)
- **Potential "functional" autoimmunity** driven by **GPCR autoantibodies** and other receptor-targeted antibodies Unlike classical autoimmune diseases that present high inflammatory markers (IL-6, TNF-α, CRP), many long COVID patients have **normal or low "classic" inflammatory indicators** yet exhibit **high-symptom burdens** (fatigue, tachycardia, brain fog, blood pressure dysregulation, etc.).

Mounting evidence suggests **autoantibodies against G-protein–coupled receptors (GPCRs)** and other regulatory receptors (ACE2, AT1R, ETAR) can functionally disrupt normal physiologic regulation without triggering a major inflammatory cascade. This leads to chronic, non-inflammatory receptor dysfunction manifesting as dysautonomia, vascular instability, and metabolic strain.

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2. Overview of Key Autoantibodies

Recent tests (often via CellTrend or similar panels) reveal multiple **functional autoantibodies**:

1. **Anti-AT1R (Angiotensin II Type 1 Receptor)**
  - **Normal Role**: Regulates blood pressure, vascular tone, fluid balance.
  - **Pathogenic Effect**: Chronic overactivation → vasoconstriction, oxidative stress; blockade → dysregulated BP control.
2. **Anti-ETAR (Endothelin-1 Type A Receptor)**

- **Normal Role**: Controls vasoconstriction, vascular remodeling.
- **Pathogenic Effect**: Leads to endothelial dysfunction, abnormal vascular tone, potential fibrotic changes.

3. **Anti–α-1-Adrenergic Receptor**
   - **Normal Role**: Modulates peripheral vasoconstriction, helps maintain vascular resistance.
   - **Pathogenic Effect**: Orthostatic intolerance, unstable blood pressure, cold extremities.
4. **Anti–β-1-Adrenergic Receptor**
   - **Normal Role**: Governs heart rate and contractility in cardiac tissue.
   - **Pathogenic Effect**: Overstimulation → tachycardia, palpitations; blockade → reduced cardiac output; overall autonomic instability.
5. **Anti–β-2-Adrenergic Receptor**
   - **Normal Role**: Smooth muscle relaxation (bronchi, vasculature), metabolic regulation.
   - **Pathogenic Effect**: Dysfunction → shortness of breath, exercise intolerance, vascular reactivity problems.
6. **Anti–Muscarinic (M3, M4, M5) Receptors**
   - **Normal Role**: Parasympathetic signaling (heart rate, glandular secretions, gut motility).
   - **Pathogenic Effect**: GI dysmotility, urinary symptoms, sweating abnormalities, cognitive/neurological changes.
7. **Anti-ACE2 (Angiotensin-Converting Enzyme 2)**
   - **Normal Role**: Converts angiotensin II to angiotensin (1–7), reduces inflammation and vasoconstriction; also the SARS-CoV-2 entry receptor.
   - **Pathogenic Effect**: Blocking ACE2 → unopposed angiotensin II → elevated blood pressure, inflammation.
8. **Anti-MAS1 (Mas Receptor)**
   - **Normal Role**: Responds to angiotensin (1–7), promoting vasodilation and organ protection.
   - **Pathogenic Effect**: Inhibiting MAS1 → pro-inflammatory, vasoconstrictive state.
9. **Anti-FGF Receptor-3**
   - **Normal Role**: Involved in cell growth, tissue repair, skeletal/neuronal tissues.
   - **Pathogenic Effect**: Interferes with tissue healing, possible neuropathic or structural changes.
10. **Anti-TSHDS-IgM** (TSH Docking Site)

- **Normal Role**: Regulates TSH receptor interactions, thyroid function.
- **Pathogenic Effect**: Typically milder, but can contribute to thyroid dysregulation and fatigue.

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3. Mechanisms of Action & How They Self-Perpetuate

3.1 Receptor Dysregulation and Immune Miscommunication

- **"Functional" Autoantibodies**
  Many are **IgG4** or other subclasses that alter receptor function (agonism/antagonism) without eliciting strong complement activation or cellular destruction.
- **Neuroendocrine Disruption**
  By binding receptors in the autonomic (adrenergic, muscarinic) or renin–angiotensin pathways (ACE2, AT1R), these autoantibodies imbalance sympathetic/parasympathetic signals—leading to persistent dysautonomia and vascular instability.
- **Chronic Th2 Bias**
  Elevated IL-4 fosters class switching to IgG4, allowing autoantibodies to persist in circulation without robust clearance.

3.2 Feedback Loops Sustaining Autoantibody Production

1. **Loss of Negative Feedback**

- Normally, β2 and muscarinic signaling dampen excessive immune activation; autoantibodies break these loops, perpetuating immune dysregulation.

2. **Molecular Mimicry & Epitope Spreading**
   - The initial SARS-CoV-2 infection or repeated exposures can trigger cross-reactivity, and ongoing tissue damage reveals new epitopes that broaden the autoimmune response.
3. **IgG4 Subclass Characteristics**
   - **Weak Complement Activation** → Minimizes antibody clearance.
   - **Low Fc Receptor Binding** → Less likely to be recognized by "cleanup" immune cells.

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4. Clinical Consequences in Long COVID

Lab findings such as **low CD8+ T cells, high IL-4, high RANTES, elevated IgG4** support a "low-inflammation but high-autoantibody" pattern. Common manifestations:

1. **Autonomic Dysfunction**
   - POTS-like symptoms, orthostatic intolerance, palpitations, HR/BP variability (due to anti-β1, β2, α1, etc.).
2. **Vascular & Endothelial Issues**
   - Anti-AT1R, ETAR, ACE2 create microcirculatory problems, potential microclotting, and endothelial dysfunction.
3. **Energy Crashes & Mitochondrial Strain**
   - Chronic receptor disruption impairs metabolic regulation, leading to post-exertional malaise.
4. **Non-Inflammatory Autoimmunity**
   - Normal/low TNF-α, IL-6, negative standard autoantibody panels (e.g., ANA) but **persistent functional impairments** (consistent with IgG4-driven dysfunction).

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5. Relevant Lab Findings & Their Interpretation

1. **High IL-4 and CCL5 (RANTES)**
   - Indicates a Th2-dominant, antibody-promoting environment.
2. **Elevated IFN-γ**
   - Ongoing T cell or NK cell activation, possibly in response to persistent viral fragments or repeated exposures.
3. **Low CD8+ T Cells**
   - Suggests T cell exhaustion, limiting effective viral/pathogen clearance and prolonging immune dysregulation.
4. **Elevated IgG4**
   - Non-inflammatory subclass that can functionally disrupt receptors.
5. **Multiple GPCR Autoantibody Positives**
   - Confirms the likelihood of a "functional" autoimmune process—especially in the context of long COVID.

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6. Downstream Factors That Perpetuate Autoimmunity

Even if GPCR autoantibodies are the **initial trigger**, **mitochondrial dysfunction, endothelial dysfunction, and microclot formation** can **amplify** or **sustain** these processes.

7.1 Mitochondrial Dysfunction

1. **DAMP (Damage-Associated Molecular Patterns) Release**
   - Dysfunctional mitochondria leak mtDNA and ROS, activating innate immune pathways (TLR9, etc.) → perpetuating immune "alert."
2. **Reduced Immune Regulation**
   - Tregs need adequate ATP; low energy states impair immune tolerance, prolonging autoantibody production.
3. **Cumulative Stress on B/T Cells**
   - Exhausted T cells (especially CD8+) fail to clear latent pathogens or address new triggers, fueling chronic immune activation.

Net Effect: Cells under metabolic stress continuously signal danger, driving ongoing B cell activation and autoantibody maintenance.

7.2 Endothelial Dysfunction

1. **Endothelial Cell Activation**
   - Damaged endothelium expresses adhesion molecules and inflammatory mediators, promoting local immune infiltration even if systemic CRP is normal.
2. **Exposure of Novel Epitopes**
   - Chronic irritation can reveal "hidden" antigenic sites on endothelial surfaces, broadening or reinforcing autoantibody targets.
3. **Vascular Instability & Autonomic Feedback**
   - BP fluctuations can reinforce hyperadrenergic states, perpetuating the cycle of receptor autoantibody signaling.
   Net Effect: Chronic endothelial damage leads to local immune overstimulation and epitope spreading, sustaining or increasing autoantibody levels.

7.3 Microclots (Fibrinaloid Microthrombi)

1. **Tissue Hypoxia & Inflammatory Signals**
   - Microclots hamper blood flow → partial hypoxia → release of IL-1β, HIF-1α signals → ongoing immune activation.
2. **Increased Vascular Shear Stress**
   - Microclots physically irritate capillary linings, causing mini "injuries" that expose new antigens or deposit immune complexes.
3. **Coagulation & Immune Crosstalk**
   - Some IgG autoantibodies may still partially activate complement or other pathways, perpetuating a **low-grade pro-inflammatory** environment.
   Net Effect: Microclots perpetuate tissue damage and local immune engagement, enhancing autoantibody production and vascular dysregulation.

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7. Additional Perpetuating Factors & Testing Before Plasmapheresis

Because **plasmapheresis** can be expensive and invasive, many clinicians ensure that **other hidden or co-factors** are addressed first to prevent **autoantibody rebound**. Areas to investigate:

1. **Chronic Infections / Latent Reactivations**
   - EBV, CMV, HHV-6, VZV; Lyme/coinfections (Babesia, Bartonella); mold toxins/mycotoxins.
   - Persistent infections or toxins drive **continuous immune stimulation**.
2. **Mast Cell Activation (MCAS)** & Allergies
   - High histamine states can keep IL-4 levels elevated, fueling IgG4.

- Testing: serum tryptase, urinary histamine metabolites, IgE panels.

3. **Gut Dysbiosis & "Leaky Gut"**
   - Chronic endotoxin (LPS) translocation fosters Th2/Th17 skew → autoimmunity.
   - Testing: comprehensive stool analysis, zonulin, lactulose–mannitol.
4. **Heavy Metals / Environmental Toxins**
   - Mercury, lead, pesticides can stress mitochondria, hamper immune regulation.
   - Testing: hair/blood/urine panels (controversial methods exist).
5. **Hormonal / Metabolic Imbalances**
   - Thyroid, adrenal (cortisol, DHEA), sex hormones (estrogen, progesterone, testosterone).
   - Dysregulated hormones can tip Th1/Th2 balance and hamper Treg function.
6. **Advanced Immune Phenotyping**
   - T cell exhaustion markers (PD-1, CTLA-4, LAG-3), NK cell function tests, inflammatory vs. regulatory cytokine panels.
7. **Microvascular & Clotting Markers**
   - Microclot detection (fibrin amyloid deposits), TEG/ROTEM, VWF/ADAMTS13 imbalance.
   - Endothelial function tests (EndoPAT, FMD).

Strategy:

- **Rule out chronic triggers** (infections, mold, toxins).
- **Stabilize hormones/nutrition**.
- **Assess gut/endothelial integrity**.
- **Confirm T cell exhaustion**.

This ensures the highest chance of **durable remission** from plasmapheresis, minimizing the risk of rapid autoantibody re-accumulation.

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8. Putting It All Together (Summary)

1. **Upstream:**
   - **GPCR/ACE2 Autoantibodies** disrupt normal autonomic, vascular, and metabolic regulation.
2. **Midstream:**
   - **Secondary Pathologies** (mitochondrial dysfunction, endothelial damage, microclots) each feed back into the immune system by releasing **DAMPs**, exposing **new epitopes**, and maintaining **chronic "danger signals."**
3. **Downstream → Upstream Feedback:**
   - Ongoing local (endothelial, microvascular) and systemic (mitochondrial, hormonal) stress signals keep B cells and T helper cells **constantly activated**, perpetuating autoantibody production.
4. **Treatment Requires**:
   - **Immunomodulation** (Thymosin Alpha-1, VIP, Treg therapy) to reduce autoantibody generation.
   - **Receptor Stabilization** (beta-blockers, ARBs).
   - **Mitochondrial & Endothelial Support** (SS-31, NAD+, microclot management).
   - **Lifestyle & Nutritional** interventions to reduce inflammatory triggers.
   - **Addressing Co-Triggers** (latent infections, mold, hormone imbalances) prior to or alongside **plasmapheresis** for a lasting result.

Treatment Strategies

Goals of This Protocol

- [ ] Lower IFN-γ and CCL5 (RANTES)
- [ ] Restore TNF-α and exhausted CD8+ T cells
- [ ] Block immune infiltration (CCR5 axis) to reduce autoantibody production
- [ ] Clear existing autoantibodies via plasmapheresis
- [ ] Build long-term tolerance through LDN, mitochondrial repair, and gut-immune restoration

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🗓️ Yearly Protocol Schedule (Week by Week)

Phase 0: CCR5 Blockade & Immune Priming

Weeks 0–4 (4 weeks)

- **Miraviroc**: 150 mg 2x/day, or 300 mg once/day if approved
  ↳ Monitor for liver enzymes, side effects
- **LDN**: Start at 1.5 mg/night, increase by 0.5 mg every 7–10 days to reach 4.5 mg
- **Melatonin**: 3–5 mg nightly
- **Butyrate or Tributyrin**: 500–1,000 mg/day with meals
- **BPC-157**: 200–500 mcg/day, SubQ or oral, 5x/week

> Purpose: Block CCL5–CCR5 infiltration pathway before plasmapheresis; activate tolerance and resolution pathways

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Phase 1: Plasmapheresis & Full Immune Reset

Weeks 4–12 (8 weeks)

- **Plasmapheresis**: 5-6 sessions
- **Rapamycin**: 3 mg once/week (e.g., Monday AM)
- **LDN**: Continue 4.5 mg nightly
- **Miraviroc**: Continue 150 mg 2x/day
- **SS-31**: 5–10 mg/day SubQ, Mon–Fri
- **BPC-157**: Continue 5x/week
- **Butyrate**: Continue
- **Melatonin**: Continue

> Purpose: Remove circulating autoantibodies after Miraviroc has reduced new production; rebuild CD8+ energy and calm chronic T cell overactivation

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Phase 2: Off Time / Reset

Weeks 12–16 (4 weeks)

- **Efgartigimod** 10 mg/kg IV weekly × 4 (start 10-14 d after last pheresis)
- **Optional IVIG bridge** 0.4 g/kg once, 1 wk before first efgartigimod dose

> Purpose:_ prevent rebound of GPCR auto-Abs while sparing B-cells.

- **Rapamycin**: Pause
- **LDN**: Continue
- **Miraviroc**: Consider stopping here unless symptoms warrant continuation
- **SS-31**: Optional continuation or pause 2–4 weeks
- **BPC-157 + Butyrate**: Continue
- **Melatonin**: Continue or pause 1 week
- **(Optional)**: Epitalon 10 mg/day x 10 days for pineal–immune reset

> Purpose: Let immune system stabilize post-phereses; assess clinical/lab changes

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Phase 3: Mitochondrial + Tolerance Rebuild

Weeks 16–24 (8 weeks)

- **LDN**: Continue 4.5 mg
- **Rapamycin**: Resume 2 mg once/week if needed
- **SS-31**: Restart or continue at 5–10 mg/day
- **BPC-157 + Butyrate + Melatonin**: Ongoing
- **Miraviroc**: Resume if RANTES is still high or infiltration symptoms persist
- **Thymosin Alpha-1 (Tα1)**
  - Dose: 1.6 mg SubQ
  - Frequency: 2× per week
  - Cycle: 8-week cycles with a 4-week break
  - Primary Effects: Lowers IgG4 production, balances Th1/Th2 responses, modulates autoantibody production.

> Purpose: Deep T cell repair, TNF-α recovery, and long-term immune balance

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Phase 4: Light Immune Tuning

Weeks 24–30 (6 weeks)

- **LDN**: Optional taper to 3 mg if stable
- **Rapamycin**: Light pulses (2 mg every 10–14 days)
- **SS-31**: Continue 3–5x/week or pause
- **Melatonin, Butyrate, BPC-157**: Continue or taper based on labs/symptoms

> Purpose: Maintain gains, prevent immune rebound, encourage tolerance

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Phase 5: Maintenance & Optional Cycles

Weeks 30–52 (5 months)

- **LDN**: Stay on if beneficial, or pulse off for 1–2 weeks every 3 months
- **Rapamycin**: Use as-needed (e.g., monthly 3 mg pulse)

- **SS-31**: Optional cycling (8 weeks on / 4 off)
- **Melatonin**: Maintain for circadian immune rhythm
- **Labs**: Recheck autoantibodies, IFN-γ, TNF-α every 8–12 weeks
- **(Optional)**: Plasmapheresis repeat if titers rise again

> Purpose: Sustain progress, prevent relapse, fine-tune as needed

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Ideal Lab Monitoring

Time-point	Key tests
Week 0	IFN-γ, TNF-α, CCL5, CBC, CMP, LFTs, GPCR panel, IgG & IgG4, CD8 flow (+ exhaustion markers), plasmablast %, lactate/pyruvate, F2-isoprostanes, microclot panel, HRV 24 h
Week 4	Same as Week 0 + miraviroc LFTs
Week 6 *(post-pheresis / pre-efgartigimod)*	GPCR panel, IgG, IgG4, CMP, albumin
Week 8 *(during efgartigimod)*	CBC, CMP, IgG, IgG4, cytokines
Week 10 *(efgartigimod nadir)*	GPCR panel, IgG, IgG4, plasmablast %, cytokines, CMP
Week 14	Same as Week 10 → **decision node** for obinutuzumab
Obinutuzumab Day 0	CBC, CMP, quantitative IgG/A/M, HBV DNA (if core +), CMV PCR (if IgG +), LDH
Obinutuzumab Day 14	CBC, CMP, infusion-reaction log
Month 3 / 6 / 12	CBC, CMP, IgG, GPCR panel, IgG4, cytokines, CD19 re-population, plasmablast %
q 8–12 wks (maintenance)	Auto-Abs, IFN-γ, TNF-α, CCL5, mito markers, symptom score

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Treatment Side-Notes:

B-Cell Testing:

A. Standard B-Cell Flow Cytometry

- **CD19, CD20**: Total B-cell count
- **CD27**: Memory B cells (CD27+) vs. naïve (CD27–)
- **CD38 High**: Plasmablasts or plasma cells (CD27++/CD38++)
- **Transitional B cells** (CD24++, CD38++)

What It Tells You

1. **B-Cell Overactivity Clues**
   - **Elevated plasmablasts** can indicate a **recent, robust antibody response**, often seen in active autoimmunity or infection.
   - High memory B cells can suggest **persistent or chronic antigenic stimulation**.
2. **Potential for Autoantibody Production**

- If memory B cells or plasmablasts are **significantly increased**, it *might* imply ongoing autoantibody generation, though not definitive.

What It *Doesn't*

- **Which specific antibodies** are being produced, or whether they're autoantibodies.
- The *function* of those B cells (some subsets could be regulatory B cells vs. pathogenic).
- Whether long-lived plasma cells (CD138+ in the bone marrow) are involved.

Bottom line: **Expanded B-cell flow** can show if you have a **B-cell hyperactivation pattern** (plasmablast expansion, large memory B-cell pool). It's not direct proof of autoantibody production but can be a good **"overactivity" gauge** for deciding on B-cell–modulating treatments.

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2. Comparing IVIG, Rituximab, and Efgartigimod for B-Cell / IgG Modulation

A. IVIG (Intravenous Immunoglobulins)

1. **Mechanism**
   - A **broad immunomodulator**: saturates Fc receptors, blocks complement activation, neutralizes certain pathogenic autoantibodies, and can upregulate inhibitory receptors on immune cells.
   - Doesn't specifically **kill** or **deplete** B cells; rather, it **interferes** with autoantibody activity and resets immune regulation *temporarily*.
2. **Uses**
   - **Autoimmune diseases** (myasthenia gravis, CIDP, etc.)
   - **Hypogammaglobulinemia** or immune deficiency
   - **Neurological autoimmunity** or severe flares (can quickly dampen autoimmune activity)
3. **Duration**
   - Effects last **3–6 weeks**; repeated infusions often needed.
   - Typically safe, but can be expensive, require IV administration, and has short-term side effects (headache, infusion reactions).
4. **Key Points**
   - **Broad** — can quickly "flood" the system with normal IgG to block pathogenic antibodies and modulate immune cells.
   - Great for **acute flares** or bridging therapy but doesn't **directly reduce** the B-cell population driving autoantibodies long-term.

B. Rituximab

1. **Mechanism**
   - A **monoclonal antibody** against **CD20** on B cells.
   - **Depletes** most circulating B cells (naïve, memory).
   - **Plasma cells** (which often lose CD20 expression) may remain, so if the disease is driven by long-lived plasma cells, additional approaches might be necessary.
2. **Uses**
   - **Rheumatoid arthritis**, **vasculitis** (ANCA-associated), **MS**, **lupus**, etc.

- Great for diseases with clear B-cell involvement (autoantibody production).

3. **Duration**
   - B-cell depletion can last **6–9 months** or more.
   - Reconstitution of B cells can begin around **4–6 months** post-infusion.
4. **Key Points**
   - **Depletes** the population that can turn into autoantibody-producing cells in the future.
   - **Doesn't** always remove existing long-lived plasma cells that no longer express CD20.
   - Typically used for **chronic** autoimmune conditions, can have infusion reactions, infection risk from immunosuppression, etc.

C. Efgartigimod (FcRn Inhibitor)

1. **Mechanism**
   - Binds the neonatal Fc receptor (**FcRn**) in endothelial and hematopoietic cells.
   - **Accelerates clearance of circulating IgG** (all subclasses, including IgG4) by blocking FcRn-mediated recycling.
   - Does **not deplete B cells**—it simply lowers the IgG "pool" for ~8-12 weeks.
2. **Uses**
   - FDA-approved for **generalized myasthenia gravis** (auto-Ab mediated).
   - Phase-2 trials in **post-COVID POTS/long-COVID dysautonomia**, immune thrombocytopenia, pemphigus.
   - Attractive as a **post-plasmapheresis "maintenance"** to prevent antibody rebound.
3. **Duration**
   - IgG falls ≈ 55-65 % after a **4-week cycle** (weekly infusions).
   - Rebounds toward baseline by **week 10-12**; repeat cycles given on clinical relapse (no fixed schedule).
4. **Key Points**
   - **Reversible & cell-sparing**: immune memory is preserved because B cells stay intact.
   - **Lower infectious risk** than rituximab; no PML or HBV re-activation signal to date.
   - Can be combined with IVIG (IVIG first, then efgartigimod) or used after plasmapheresis.
   - Limitations: effect is temporary; repeat cycles incur cost; transient IgG drop can raise mild URTI/UTI rate.

Safety Profile

Factor / Drug	IVIG	Rituximab	Efgartigimod
Serious infection risk	< 2 %	5–10 % / yr	4–6 % per cycle (mostly mild)
HBV re-activation / PML	None	**Yes** (rare but documented)	None reported
Infusion reactions	Mild (HA, chills)	5–10 % severe IRR	Mild; ≤ 5 % HA, flushing
Immunoglobulin effect	Adds IgG	May ↓ IgG long-term	IgG ↓ 55–65 % transiently
B-cell depletion	✗	✓	✗
Recovery / reversibility	3–6 wks	6–12 mo	8–12 wks
Ideal niche	Acute flare or bridge	Long-term B-cell overdrive	Post-pheresis antibody rebound prevention

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3. B-Cell / IgG-Targeted Treatment Summaries

Aspect	IVIG	Rituximab	Efgartigimod
Approach	Functional immunomodulation; blocks Ab/complement	**Depletes B cells** ($CD20^+$)	**Accelerates IgG clearance** via FcRn blockade
Speed of Onset	Hours–days (rapid flare control)	Weeks to full B-cell nadir	IgG drop within 1–2 weeks
Longevity	3–6 wks per infusion	6–9 mo (or longer)	8–12 wks per 4-dose cycle
Target	All IgG subclasses + broad immune modulation	Naïve & memory B cells (not plasma cells)	Circulating IgG (all subclasses)
Ideal Use Case	Acute flare, bridging, IgG deficiency	Chronic autoimmunity with proven B-cell overdrive	Preventing auto-Ab rebound post-pheresis or IVIG
Downsides	Temporary; IV access; cost	Infection / re-activation risks; does not remove plasma cells	Requires repeat cycles; mild ↑ in URTI/UTI; cost

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Treatments for ACE2/GPCR autoantibodies

If testing confirms **pathogenic functional autoantibodies** (e.g., anti-ACE2, β2, muscarinic), the following must be considered:

1. **IVIG or Plasmapheresis**
   - **Plasmapheresis** (therapeutic plasma exchange) is **more effective than IVIG** in cases where pathogenic IgG4 autoantibodies cause receptor dysfunction.
2. **ARBs (Losartan, Telmisartan)**
   - Block **excess Ang II** effects in the setting of dysfunctional ACE2 activity.
3. **Beta-Blockers**
   - Useful if **sympathetic overdrive** or tachyarrhythmias are predominant.
4. **Peptides**
   - **Thymosin Alpha-1** to modulate autoantibody production and support T-cell function.
   - **VIP (Vasoactive Intestinal Peptide)** for anti-inflammatory, mast-cell stabilization, and immunomodulation.
   - **SS-31 (Elamipretide)** to aid **mitochondrial repair** and reduce oxidative stress.

Note on IgG4 Autoantibodies:
Many GPCR autoantibodies that cause functional blockade or overstimulation can be **IgG4**, which does not trigger a classic inflammatory cascade. Clinical improvement often requires **immunomodulatory** (rather than purely anti-inflammatory) therapies.

- **IgG4 Autoantibodies Are Often "Functional" Rather Than Destructive**
  - Unlike **IgG1 or IgG3**, which usually trigger **inflammatory immune responses** (complement activation, cell-mediated cytotoxicity), **IgG4 autoantibodies** tend to interfere with receptor signaling **without causing overt inflammation**.
  - This means that many **classic autoimmune treatments (e.g., steroids, TNF blockers, IL-6 inhibitors)** may not be effective.
- **Inflammatory Markers May Be Normal Despite Autoimmune Activity**
  - Many long COVID patients with GPCR autoantibodies **do not have elevated IL-6, TNF-α, or CRP**, which are hallmarks of inflammatory autoimmunity.
  - Instead, they present with **dysautonomia, vascular issues, and metabolic dysfunction**, which are more consistent with **receptor dysfunction rather than immune cell attack**.
- **Treatment Must Focus on Receptor Stabilization & Immunomodulation**

- Since IgG4 does not trigger inflammation directly, therapies must **reduce autoantibody production (e.g., Thymosin Alpha-1, IVIG, Plasmapheresis)** and **stabilize affected receptors (e.g., ARBs, beta-blockers, VIP peptide)**.
- Standard immunosuppressive drugs (e.g., prednisone, methotrexate) may be **ineffective or even counterproductive** because they do not address **functional autoimmunity**.

- **IVIG and Treg-Based Therapies in IgG4 Autoantibody Conditions: Effectiveness Considerations**
  - IVIG and therapies that promote **regulatory T cell (Treg) function** (e.g., IL-2, rapamycin, low-dose naltrexone) are commonly used in **classical autoimmune diseases**, but their effectiveness in **IgG4-mediated autoimmunity** can be variable due to the unique **non-inflammatory** and **functional** nature of IgG4 autoantibodies.

Background Information

Autoantibodies and Disease States

ME/CFS and Neurological Disorders

- **High Levels of Autoantibodies:**
  Chronic fatigue syndrome (ME/CFS), as well as certain neurological or psychiatric conditions (e.g., schizophrenia, bipolar disorder), have been associated with elevated levels of autoantibodies. Notably, these include autoantibodies targeting β2-adrenergic receptors (ADRB2) and muscarinic acetylcholine receptors.
- **Symptom Overlap:**
  Patients often experience muscle weakness, cognitive impairment (“brain fog”), autonomic dysfunction (e.g., irregular heart rate, blood pressure instability), and profound fatigue.
- **Potential Mechanisms:**
  - **Molecular Mimicry:** In susceptible individuals, infections or inflammatory triggers can result in immune cross-reactivity with host receptors.
  - **Autonomic Dysregulation:** Impaired receptor signaling can degrade the body’s ability to maintain stable heart rate, blood pressure, and other vital functions.

Autoimmune Autonomic Syndromes

- **Fibromyalgia and POTS:**
  Similar to ME/CFS, fibromyalgia and postural orthostatic tachycardia syndrome (POTS) frequently exhibit autoantibodies against G-protein coupled receptors (GPCRs).
- **Key Symptoms:**
  - **Fatigue and Cognitive Issues:** Challenges such as brain fog and concentration problems.
  - **Dysregulated Autonomic Responses:** Heart palpitations, dizziness upon standing, temperature regulation issues, and gastrointestinal disturbances.
- **Clinical Significance:**
  These findings suggest an overarching pattern in which autoantibodies disrupt GPCR function, perpetuating chronic symptom burdens in diverse patient populations.

SARS-CoV-2 and the Immune Dysregulation Cascade

Spike Protein Interaction and ACE2 Binding

1. **Primary Interaction:**
   - The SARS-CoV-2 spike protein binds to the ACE2 receptor via its receptor-binding domain (RBD).
   - After binding, the spike protein undergoes cleavage at the furin site by host proteases (e.g., TMPRSS2), facilitating viral entry (fusion and uptake).
2. **Role of ACE2 Autoantibodies:**
   - Autoantibodies can be generated against ACE2, potentially through molecular mimicry.
   - These may interfere with normal ACE2 receptor function and exacerbate immune dysregulation.
   - However, the virus itself **does not** target ACE2 autoantibodies for entry; it directly binds the ACE2 receptor.

Bridging Spike-ACE2 Interaction to GPCR Autoimmune Disorders

1. **Triggering Immune Dysregulation:**
   - **Molecular Mimicry:** Structural similarities between SARS-CoV-2 proteins and host GPCRs (e.g., β2-adrenergic, muscarinic receptors) may cause cross-reactive autoantibody production.
   - **Release of Autoantigens:** Infection and cell damage can release intracellular components into an inflammatory milieu, promoting development of new autoantibodies.
2. **Upstream Immune Drivers and Downstream Effects:**
   - **Upstream Signals:**
     - Pro-inflammatory cytokines, such as **IFN-γ** and **IL-4**, can alter immune responses (e.g., promoting IgG4 class switching).
   - **Downstream Consequences:**
     - Autoantibodies against GPCRs can lead to persistent receptor activation/blockade, manifesting in cardiovascular issues, chronic fatigue, and autonomic dysfunction.
     - Elevated **IgG4** levels, while sometimes reducing inflammation, may also dampen immune clearance against certain pathogens.
3. **Nanoparticle Considerations in mRNA Vaccines:**
   - Lipid nanoparticles (LNPs) deliver mRNA safely, but concerns persist about potential oxidative stress and inflammation.
   - In vulnerable individuals, heightened or dysregulated immune responses could intersect with existing autoimmune tendencies.

Bridging the Gap: From Spike Protein Binding to GPCR Autoimmunity

1. **Initial Viral Entry:**
   - Spike binds ACE2, enabling SARS-CoV-2 to infect host cells.
2. **Immune Activation and Inflammation:**
   - The body mounts a strong innate and adaptive response, which can include cytokine release and tissue damage.
3. **Potential Autoimmune Trigger:**
   - Intense inflammation may facilitate molecular mimicry or increased presentation of self-antigens, fueling an autoimmune cascade.
4. **Production of Autoantibodies:**
   - β2-adrenergic and muscarinic receptor autoantibodies can emerge, disrupting normal signaling in autonomic pathways.
5. **Chronic Immune Dysregulation:**
   - Ongoing immune hyperactivation leads to reduced tolerance, sustained inflammation, and symptomatic presentations such as POTS or ME/CFS.

Comprehensive Insights on POTS, IgG4 Class Switching, and the Vagus Nerve

Below is an expanded overview integrating the role of immunoglobulin subclass switching (especially IgG4), cytokines like TNF-α and IFN-γ, and the modulatory function of the vagus nerve.

1. Overview of POTS and Long COVID

- **Overlap in Symptoms:**
  Both POTS and long COVID can include heart rate anomalies, dizziness, fatigue, and cognitive dysfunction ("brain fog").
- **Autoantibody Role:**
  Autoantibodies (e.g., targeting adrenergic or muscarinic receptors) frequently appear in these syndromes, causing or intensifying dysautonomia.

2. Autoantibodies: Adrenergic, Muscarinic, and ACE2

- **Adrenergic Receptor Autoantibodies:**
  - **Alpha-1** receptor autoantibodies impair vasoconstriction; **beta-1 and beta-2** disrupt normal heart rate control.
- **Muscarinic Receptor Autoantibodies:**
  - Especially M2 and M3 involvement—leading to heart rate irregularities, GI motility issues, and sweating abnormalities.
- **ACE2 Autoantibodies:**
  - Can undermine vascular homeostasis, blood pressure regulation, and possibly worsen post-viral autonomic dysfunction.

3. Mechanisms of IgG4 Class Switching

- **Repeated Antigenic Stimulation (e.g., Multiple mRNA Vaccinations):**
  - Documented shifts toward IgG4 production have been observed, particularly after the third or fourth vaccine dose.
- **Immune Tolerance vs. Protective Response:**
  - **High IgG4** can act tolerogenically (beneficial in allergies), but may also reduce the immune system's effectiveness in clearing viruses or abnormal cells if excessively elevated.
- **Potential Impact on Disease:**
  - Persistent IgG4 antibodies could influence chronic inflammation, autoimmunity, or re-infections, though more research is needed to confirm long-term clinical outcomes.

4. Pro-Inflammatory Cytokines: TNF-α and IFN-γ

- **TNF-α in Chronic Inflammation**
  - Often elevated in POTS, autoimmune disorders, and prolonged viral syndromes.
  - Heightened TNF-α correlates with more severe fatigue, autonomic instability, and potential tissue damage.
- **IFN-γ**
  - A central cytokine in Th1-driven immune responses; chronic elevation may promote ongoing inflammation and autoantibody production.
  - Can act synergistically with other cytokines to maintain a feedback loop of immune activation.

5. Vagus Nerve Dysfunction and the Cholinergic Anti-Inflammatory Pathway

1. **Cholinergic Anti-Inflammatory Pathway (CAP):**

- The vagus nerve, through acetylcholine release, helps inhibit excessive pro-inflammatory cytokine release (including TNF-α).

2. **Dysfunction Exacerbates Disease:**
   - Impaired vagal tone can lead to unchecked inflammation, contributing to POTS, long COVID symptoms, and possibly autoantibody proliferation.
3. **Neuromodulation Treatments:**
   - **Vagus Nerve Stimulation (VNS)** has shown promise in clinical settings to rebalance autonomic function and reduce inflammation.

6. Diet, Exercise, and Autoimmune Flare-Ups

- **Dietary Influences:**
  - Pro-inflammatory diets (high in refined sugars, processed foods) may aggravate autoimmune activity.
  - Anti-inflammatory diets can help modulate systemic inflammation and may ease symptom severity.
- **Exercise Stress:**
  - Intense workouts can exacerbate autonomic dysregulation and post-exertional malaise (PEM).
  - Gentle, graded exercise (swimming, recumbent biking, yoga) is often recommended to maintain physical conditioning without triggering flares.

7. Therapeutic Approaches

1. **Immunomodulatory Treatments:**
   - **IV Immunoglobulin (IVIG)**: May reduce pathogenic autoantibodies, though availability and cost are limiting factors.
   - **Immunosuppressants** (e.g., rituximab, corticosteroids): Suppress B-cell activity but raise infection risks.
2. **Medications for Autonomic Control:**
   - **Beta Blockers, Ivabradine**: Assist with heart rate regulation in POTS.
   - **Fludrocortisone, Midodrine**: Improve orthostatic tolerance via volume expansion and vasoconstriction.
3. **Lifestyle Interventions:**
   - **Hydration and Electrolytes**: Adequate fluid and salt intake to stabilize blood pressure.
   - **Stress Management**: Mindfulness, meditation, and moderate physical activity to enhance vagal tone.
4. **Addressing the Cell Danger Response (CDR):**
   - Chronic activation of a protective “cell danger” state can trap the body in persistent inflammation. Techniques that restore metabolic homeostasis (rest, targeted supplements, VNS) may help break this cycle.altered monocyte populations), further cementing the autoimmune phenotype.

IVIG (Intravenous Immunoglobulin) and IgG4 Autoantibodies

🔹 **How IVIG Works:**

- IVIG contains pooled **polyclonal immunoglobulins (mostly IgG1 and IgG3)** from thousands of donors.
- It can work via multiple mechanisms:
  - **Fc receptor blockade** to reduce autoantibody effects.
  - **Enhancement of regulatory pathways** (increasing IL-10, promoting Tregs).
  - **Competing with autoantibodies for target binding** (neutralization).
  - **Increasing IgG catabolism** (accelerated clearance of pathogenic IgG subclasses).

🔹 **Effectiveness in IgG4-Mediated Disease: Sometimes effective** when autoantibody levels are very high and functional disruption is severe. **Less consistently effective compared to IgG1/IgG3-driven autoimmune diseases**, because:

- **IgG4 does not activate complement or Fc receptor signaling**—which are key targets of IVIG.
- **IVIG may not efficiently clear IgG4 autoantibodies**, as IgG4 has a longer half-life and different clearance pathways compared to IgG1/IgG3.
- **The underlying problem in IgG4-mediated disease is often receptor dysfunction**, not inflammatory destruction.

🛠 When to Consider IVIG for IgG4 Autoantibodies?

- If there are **high titers of pathogenic IgG4 autoantibodies** and **severe functional impairment** (e.g., profound dysautonomia, refractory vascular dysfunction).
- If other **immunomodulatory therapies (Thymosin Alpha-1, peptides) fail**.
- If **some IgG1/IgG3 autoantibodies are present alongside IgG4**, IVIG may still help.

🔹 **Alternative to IVIG in IgG4 Autoimmunity:**

- **Plasmapheresis** (therapeutic plasma exchange) is **more effective than IVIG** in cases where pathogenic IgG4 autoantibodies cause receptor dysfunction.
- **Targeted B-cell depletion (Rituximab, anti-CD19 therapies)** may be needed for persistent high IgG4 autoantibody production.

What Is a COVID-19 S1-Immune Subset Test?

The S1 Immune Subset looks for the presence of SARS COV 2-S1 protein in monocytes resulting from either the COVID-19 vaccine or natural
infection. This test also includes CD3, CD4 and CD8 % and absolute count in addition to monocyte subset percentage with and without S1 protein in
all classical, non-classical, and intermediate monocytes.

The S1 Immune subset simply tells if the S1 is in your system and if the present most likely cause for the long hauler symptoms.

Post-acute sequelae SARS-CoV-2 infection (PASC) is a disabling and sometimes debilitating condition that occurs in 10%-30% of individuals infected
by SARS-CoV-2 and has recently been proposed to cause neurologic symptoms in 30% of those infected.

Recently Dr. Patterson and his team identified characteristic immune cell subset abnormalities that accompanied the unique cytokine/chemokine
profile. They also found kinetic differences in the proportions of monocyte subsets in severe cases and PASC, as well as the presence of SARS-CoV-2
protein unaccompanied by corresponding viral RNA in CD14lo, CD16+ monocytes in PASC patients up to 16 months post-acute SARS-CoV-2
infection.

The predominant immune cell abnormality they observed in their work was elevations in monocyte subsets. Monocyte subpopulations are divided
into 3 phenotypes, and they are functionally distinct from each other.

Classical monocytes: Exhibit the CD14++, CD16- phenotype. The classical monocytes express high levels of the ACE-2 receptor, the putative
receptor for SARS-CoV-2. classical monocytes express low levels of the chemokine receptors CX3R1 and CCR5.

Intermediate monocytes: Exhibit a CD14+, CD16+ phenotype, express very little ACE-2 receptor, and express high levels of CCR5.

Non-classical monocytes: Exhibit CD14lo, CD16+ phenotype, express very little ACE-2 receptor, express high levels of CX3R1.

Identifying the immune subset population may provide information in understanding symptoms associated with inflammatory phenotype of these
senescent nonclassical monocytes and assist in the treatment of PSAC.

T LYMPHOCYTE SUBPOPULATION

CD4+T and CD8+T play a vital role in maintaining immune function and viral clearance in the body. It has been reported that CD4+T and CD8+T
counts significantly decreased in COVID-19 patients. Several studies revealed that CD3+ T cells, CD4+ T cells, CD8+ T cells, and natural killer cells
were significantly decreased in patients with COVID-19. These patients had a relatively slight decrease in CD4+ T cells but a severe decrease in CD8+
T cells. A significantly elevated CD4/CD8 ratio was observed in COVID-19 patients. T-cell subset counts were related to the severity and prognosis of
COVID-19, suggesting that the counts of CD8+ T and CD4+ T cells can be used as diagnostic markers of COVID-19 and predictc
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Results must be interpreted together with other clinical information available to the physician.

Test method:

This test is based on flow cytometry methodology and involves following CPT codes: 88184, 88185, 88188, 86356.

T and NK Cell Additional Tests Needed for Functional Confirmation

Flow cytometry provides a **static snapshot** of T and NK cell states. To confirm **functional exhaustion**, additional tests may be needed:

1. **Proliferation Assays**
   - Stimulate T cells with antigens or mitogens (PHA, anti-CD3/CD28) and measure **Ki-67 expression** or **BrdU uptake**.
2. **Cytokine Production Assays (ELISA or Intracellular Staining)**
   - Stimulate with PMA/Ionomycin and check for **IFN-γ, TNF-α, IL-2 production**.
3. **Mitochondrial Function Testing in T Cells**
   - **Seahorse Metabolic Assay** (Measures ATP production, glycolysis vs. oxidative phosphorylation).
   - **ROS Levels & Mitochondrial Membrane Potential** (Flow cytometry-based assays).
   flow cytometry can identify markers of T cell exhaustion **(e.g., PD-1, CD57, LAG-3, TIM-3).**
   But functional exhaustion requires additional tests** (proliferation, cytokine response, metabolic function).

Citations and Sourcing (Incomplete)

Cytokine Connection to Long Covid (Paterson and Team)

- IL-6 is a pro-inflammatory cytokine whose major function is differentiation of B-cells into plasma cells and IgG production [Turner, et al.,] IL-6 has been associated with oxidative stress, inflammation, endothelial dysfunction, and thrombogenesis [Patterson, et al., Front. Immunol.; Hou, et al., Lee, et al., Roldan, et al., Wassmann, et al.,]. IL-6 has been found to be elevated in severe COVID-19 patients and in chronic COVID patients. [Patterson, et al., IJID 2020; Patterson, et al., Front. Immunol. 2021]. IL-6 has also been demonstrated to play a role in inflammatory pain [Zhou, et al., Zhang, et al.,] and inhibitors of IL-6 or its receptors may aid in pathological pain management. [Zhou, et al.,]

- IFN-gamma: Interferon-gamma is a pro-inflammatory cytokine associated with anti-viral action, activation of macrophages, increasing neutrophil and monocyte function and MHC-1 and MHC-11 expression on cells. [Monastero, et al., Turner, et al.,] INF-gamma has been found to be elevated in chronic COVID patients. [Patterson, et al., IJID 2020 Patterson, et al., Front. Immunol. 2021].
- IL-2 is responsible for proliferation and activation of T cells, B cells and NK cells [Turner, et al.,]
- IL-4: The interleukin 4 is a cytokine that induces differentiation of naive helper T cells to Th2 cells. Upon activation by IL-4, Th2 cells subsequently produce additional IL-4 in a positive feedback loop. IL-4 is produced primarily by mast cells, Th2 cells, eosinophils, and basophils.
- CXCL8 (IL-8): Interleukin 8 is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells. Endothelial cells store IL-8 in their storage vesicles, the Weibel-Palade bodies. In humans, the interleukin-8 protein is encoded by the CXCL8 gene.
- IL-10 has been found to be elevated in chronic COVID patients. [Patterson, et al., Front. Immunol. 2021]. It has been observed that long-hauler patients demonstrate an elevation in IL-2 and IFN-gamma and a reduction in CCL4. In the context of a viral infection, elevation of these cytokines would induce the activation of effector T cells with pro-inflammatory properties and the ability to mount an effective immune response [Patterson, et al., Front. Immunol.2021]
- IL-13 is a cytokine produced by Th2 cells, NK cells, mast cells, basophils and is a mediator of allergic inflammation and a key regulator of IgE synthesis, mucus hypersecretion, and airway hyperresponsiveness. [Rael et al., Gulati, et al.,]
- GM-CSF is produced by T cells, macrophages and fibroblasts and stimulates production of granulocytes, monocytes, and eosinophils [Turner, et al.,]. GM-CSF has been found to be reduced in chronic COVID patients. [Patterson, et al.,Front. Immunol. 2021].
- CCL3 is involved in macrophage and NK-cell migration as well as T-cell dendritic cell interactions [Sokol, et al.,] CCL3 has been found to be elevated in chronic COVID patients. [Patterson, et al., Front. Immunol. 2021].
- CCL4 has been found to be reduced in chronic COVID patients. [Patterson, et al., Front. Immunol. 2021] CCL4 is involved in macrophage and NK-cell migration as well as T-cell/dendritic cell interactions [Sokol, et al.,]. CCL4 signals through the receptor CCR5 to attract T cells to the site of inflammation and depending on the immune context, can recruit differentially activated T cells [Patterson, et al., Front. Immunol.; Liu, et al; Mukaida, et al.,]
- CCL5 is involved in macrophage and NK-cell migration as well as T-cell/dendritic cell interactions [Sokol, et al.,] CCL5 levels have been found to be highly elevated in severe COVID-19 patients, but not in chronic COVID patients. [Patterson, et al., Front. Immunol. 2021; Patterson, et al., IJID 2020)
- VEGF has been found to be elevated in chronic COVID patients. [Patterson, et al., Front. Immunol. 2021]. The presence of VEGF in inflammation neuropathies such as CIOP, GBS and vascular neuropathy may indicate a potential relation with vascular involvement. [Nobile-Orazio, et al.,] VEGF has been shown to be elevated in POEMS but no other monoclonal gammopathies and can be elevated in immune-mediated neuropathies.
- TNF-alpha is a pro-inflammatory cytokine, associated with phagocyte cell activation and endotoxic shock. [Turner, et al.,) TNF-alpha has been shown to play a role in the process of pathological pain. [Zhang, et al.,]
- Soluble CD40 ligand (sCD40L) is contained in platelet granules and thus its presence in the blood is a marker of platelet activation. By interacting with CD40, which is found on endothelial and smooth muscle cells, sCD40L may trigger the release of inflammatory mediators, lead to increased activity of matrix metalloproteinases, and activate the coagulation cascade.
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Long Covid/Post Vaccine Autoimmunity

2021-Autoantibodies-as-Endogenous-Modulators-of-GPCR-Signaling: Autoantibodies (AAs) targeting G-protein-coupled receptors (GPCRs) are associated with various diseases and can modulate receptor signaling, though their molecular mechanisms are not well understood. Most GPCR AAs act as allosteric modulators, influencing receptor signaling and trafficking, with potential implications for both disease pathology and therapeutic antibody development. While biopharmaceuticals targeting GPCRs remain challenging due to receptor structure constraints, emerging antibody-based therapies show promise for selectively modulating GPCR activity.

- Skiba, Meredith A., and Andrew C. Kruse. "Autoantibodies as endogenous modulators of GPCR signaling." *Trends in pharmacological sciences* 42.3 (2021): 135-150.

2022-Nature-Communications: The study investigates the role of autoantibodies targeting G protein-coupled receptors (GPCRs) and renin-angiotensin system (RAS)-related molecules in COVID-19 severity. A cross-sectional analysis of 246 individuals revealed that patients with moderate and severe COVID-19 had significantly higher levels of these autoantibodies than healthy controls or those with mild disease.

- Cabral-Marques, Otavio, et al. "Autoantibodies targeting GPCRs and RAS-related molecules associate with COVID-19 severity." *Nature Communications* 13.1 (2022): 1220.

2023-The 4th International Symposium on Autoantibodies Targeting G Protein-Coupled Receptors (GPCRs) highlighted the evolving role of these autoantibodies in autoimmune diseases, COVID-19, post-COVID syndrome, and other pathological conditions. Researchers discussed how functional autoantibodies targeting GPCRs influence disease mechanisms, immune regulation, and potential therapeutic interventions, with particular focus on rheumatic, neurological, and cardiovascular diseases. The symposium emphasized the need for standardized methodologies to study these autoantibodies, as well as their potential as biomarkers and therapeutic targets in autoimmune and inflammatory conditions.

- Cabral-Marques, Otávio, et al. "Autoantibodies targeting G protein-coupled receptors: An evolving history in autoimmunity. Report of the 4th international symposium." *Autoimmunity reviews* 22.5 (2023): 103310.

2024-G-PROTEIN-COUPLED RECEPTORS ANTIBODIES HAVE SUBSTANTIAL VASOREGULATIVE IMPLICATIONS IN LONG-COVID: GPCR antibodies are present in Long-COVID and show various vascular implications in terms of vasorelaxation as seen in lower aortic systolic and diastolic blood pressure as well as in an amelioration of FMD

- Seibert, Felix, et al. "G-PROTEIN-COUPLED RECEPTORS ANTIBODIES HAVE SUBSTANTIAL VASOREGULATIVE IMPLICATIONS IN LONG-COVID." *Journal of Hypertension* 42.Suppl 1 (2024): e129.

Plasmapheresis as a therapy for autoimmunity in Long Covid Patients

2021-Case Report: Therapeutic and immunomodulatory effects of plasmapheresis in long-haul COVID----This case report describes a 68-year-old male with severe long-haul COVID symptoms, including lung opacity, extreme fatigue, cognitive impairment, and loss of smell, who underwent therapeutic plasma exchange (TPE). TPE led to a significant reduction in inflammatory macrophages and an increase in cytotoxic CD8+ T cells and NK cells, while also attenuating G-protein-coupled receptor (GPCR) autoantibodies, which are implicated in long-COVID pathology, suggesting a potential mechanism for symptom relief.

- Kiprov, Dobri D., et al. "Case Report: Therapeutic and immunomodulatory effects of plasmapheresis in long-haul COVID." *F1000Research* 10 (2022): 1189.

2023-Clinical improvement of Long-COVID is associated with reduction in autoantibodies, lipids, and inflammation following therapeutic apheresis. :----- Investigates the effects of therapeutic apheresis in Long-COVID patients, demonstrating a significant reduction in autoantibodies, lipids, and inflammatory markers following treatment, which correlates with clinical improvement.

- Achleitner, Martin, et al. "Clinical improvement of Long-COVID is associated with reduction in autoantibodies, lipids, and inflammation following therapeutic apheresis." *Molecular Psychiatry* 28.7 (2023): 2872-2877.

2024-A Single-Center Pilot Study of Therapeutic Apheresis in Patients with Severe Post-COVID Syndrome: ----- pilot study evaluated therapeutic apheresis in 20 patients with severe post-COVID syndrome, finding a significant reduction in autoantibodies targeting beta-adrenergic and muscarinic neurotransmitter receptors. Most patients experienced symptom relief, particularly in fatigue, physical activity restrictions, myalgia, post-exertional malaise, and concentration issues.

- Achleitner, Martin, et al. "Clinical improvement of Long-COVID is associated with reduction in autoantibodies, lipids, and inflammation following therapeutic apheresis." *Molecular Psychiatry* 28.7 (2023): 2872-2877.