Mechanisms of Long COVID – the medical hypotheses

Effect of SARS-CoV-2 itself

- The virus is still harboured in a reservoir organ which is sheltered from the immune system.
- SARS-CoV-2 is a superantigen
- Delayed viral clearance due to immune exhaustion.
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- The virus has been reverse-transcribed and integrated into our DNA
- Antibody dependent enhancement (ADE)

Direct after-effects of SARS-CoV-2

- Tissue damage from acute COVID-19.
- Persistent excess inflammation
- Overactivation of the renin-angiotensin system (RAS)
- Endothelial dysfunction, vascular injury and hypercoagulation

Indirect effects of SARS-CoV-2

- Induction of autoimmune conditions through molecular mimicry
- Mitochondrial dysfunction
- Reactivation of other dormant viruses
- Microbiome alteration
- Demyelination in brain and nervous system

Contributory factors

- Genetics
- Diet and malnutrition
- Lack of exercise and physical deconditioning due to immobility while ill
- Poor methylation/high homocysteine

Viral reservoirs

- Viral reservoirs are anatomical sites in which caches of **virus-infected cells remain hidden from immune elimination.** Here, viruses accumulate and persist, with plasma levels below the level of conventional detection. Once the initial infection has resolved, those individuals harbouring viral reservoirs are likely to test negative for the virus.
- The seeding of viral reservoirs occurs early and systemically, especially within gut-associated lymphoid tissue (GALT).
- Where the immune system is dysfunctional or distracted, the **infection in viral reservoirs is reactivated. Reactivation implies that reservoir-infected cells producing viral RNA contain an intact viral genome capable of producing an infectious virus.**

(https://www.nature.com/subjects/viral-reservoirs; Neurath MF, et al. Gut as viral reservoir: lessons from gut viromes, HIV and COVID-19 Gut 2021;70:1605-1608; Colón-Thillet, R., et al. Optimization of AAV vectors to target persistent viral reservoirs. Virol J, 2021; 18, 85; https://www.nature.com/articles/s41592-021-01145-z; Busman-Sahay K et al; Eliminating HIV reservoirs for a cure: the issue is in the tissue, Current Opinion in HIV and AIDS: July 2021 - Volume 16 - Issue 4 - p 200-208)

Viral reservoirs and SARS-CoV-2 reactivation

- There may be viral reservoirs containing RNA and other components from the SARS-CoV-2 virus that persist in the body and keep the immune system on high alert. These reservoirs might continuously stimulate systematic inflammation, despite a negative COVID test.
- Recent studies have found **SARS-CoV-2 reservoirs in Long COVID patients in multiple organs including the brain, lung, myocardium, lymphoid tissue (gut, lymph nodes and spleen) but also in the central nervous system**. (Matschke, J., et al. (2020). Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. Lancet Neurol. 19, 919–929; Gaebler, C. et al. (2021). Evolution of antibody immunity to SARS-CoV-2. Nature 591, 639–644)
- There have been **several reports of relapse or reactivation of SARS-CoV-2 in recovered COVID-19 patients**. (https://www.reuters.com/article/us-health-coronavirus-southkorea/south-korea-reports-more-recovered-coronavirus-patients-testing-positive-again-idUSKCN21V0JQ; https://health-desk.org/articles/what-do-we-know-about-covid-19-reinfection-and-reactivation)
- There is concern that in these reservoirs the virus may be mutating, producing a different variant to which the individual is not immune, hence causing reinfection. Viral persistence and its associated genetic mutations could be capable of provoking anti-viral 'antibody waves', leading to immune exhaustion. (Ramakrishnan RK, et al. Unraveling the Mystery Surrounding Post-Acute Sequelae of COVID-19. Front Immunol. 2021 Jun 30;12:686029)
- Long COVID patients who were asymptomatic during acute COVID-19 disease and who experience relapsing/remitting chronic symptoms may be more likely to harbour persistent reservoirs of SARS-CoV-2 in tissue. (Proal AD & VanElzakker MB. Long COVID or Post-acute Sequelae of COVID-19 (PASC): An Overview of Biological Factors That May Contribute to Persistent Symptoms. Frontiers in microbiology. 2021; 12, 698169)
- The literature is replete with examples of single-stranded RNA virus persistence, spanning decades of research on samples obtained from living human patients, autopsy studies, and animal studies. (Proal AD & VanElzakker MB. Long COVID or Post-acute Sequelae of COVID-19 (PASC): An Overview of Biological Factors That May Contribute to Persistent Symptoms. Frontiers in microbiology. 2021; 12, 698169)

SARS-CoV-2 is a Superantigen

- Superantigens (SAgs) are a group of microbial proteins that interact with the immune system in a unique manner. They have in common an extremely **potent stimulatory activity for CD4+, CD8+ and other T lymphocytes** by a unique mechanism: cross-linkage of variable parts of the T-cell receptor (TCR) with MHC class II molecules on accessory or target cells, inducing massive cytokine release, toxic shock, immunosuppression and/or life-threatening autoimmune responses. **They escape normal antigen processing** by antigen presenting cells (APCs) and can directly bind to the T cell receptor (TCR). Pathogens producing superantigens include Epstein-Barr virus, the retroviruses human endogenous retrovirus (HERV) and human immunodeficiency virus (HIV), and Ebola virus. Antibodies may themselves be SAgs and can activate up to 100% of T cells. (Fleischer B. Superantigens. APMIS. 1994 Jan;102(1):3-12; Us D. Viral süperantijenler [Viral superantigens]. Mikrobiyol Bul. 2016 Jul;50(3):491-504; Jacobs JJL. Persistent SARS-2 infections contribute to long COVID-19. Med Hypotheses. 2021; 149: 110538)
- Superantigens are known to induce the cytokine storm through polyclonal T cell activation and are thought to be operative in severe COVID-19. In most patients down-regulation of superantigen responses occurs naturally but where this fails, the virus could reproduce in the body, especially where the immune response is relatively weak. (Jacobs JJL. Persistent SARS-2 infections contribute to long COVID-19. Med Hypotheses. 2021; 149: 110538)

SARS-CoV-2 as a Superantigen in Long COVID

- Some of Long COVID may be due to a persistent systemic infection, as indicated by continued viral RNA shedding. It is thought that **as a superantigen, in Long COVID patients the virus overstimulates anti-viral immune responses and thereby induces negative feedback loops that allow the virus to persist.** (Jacobs JJL. Persistent SARS-2 infections contribute to long COVID-19. Med Hypotheses. 2021 Apr;149:110538)
- Multisystem Inflammatory Syndrome in Children (**MIS-C**) symptoms and the associated laboratory values strongly resemble toxic shock syndrome, an escalation of the cytotoxic adaptive immune response triggered upon the binding of pathogenic superantigens to MHCII molecules and T cell receptors (TCRs). These data suggest that the SARS-CoV-2 Spike protein may act as a superantigen to drive the development of MIS-C as well as cytokine storm in adult COVID-19 patients. (Cheng MH, et al. An insertion unique to SARS-CoV-2 exhibits superantigenic character strengthened by recent mutations. bioRxiv [Preprint]. 2020 May 21:2020.05.21.109272)

Delayed viral clearance due to immune exhaustion, or lymphopaenia

- **Persistent SARS-CoV-2 viral shedding is associated with reduced numbers of adaptive immune cells** (i.e. B and T cells) (Liu B, et al. Reduced numbers of T cells and B cells correlates with persistent SARS-CoV-2 presence in non-severe COVID-19 patients. Sci Rep. 2020;10(1): 17718).
- A study showed that **viral persistence was associated with significantly lower levels and slower generation of antibodies** (viral receptor-binding domain (RBD)-specific IgA and IgG), i.e. an absence of robust protective humoral immune response (Hu F, et al. A compromised specific humoral immune response against the SARS-CoV-2 receptor-binding domain is related to viral persistence and periodic shedding in the gastrointestinal tract. Cell Mol Immunol. 2020;17(11):1119–1125).
- Immune exhaustion is defined as the dysfunction of antigen-specific immune cells due to prolonged antigen stimulation. This exhaustion of anti-viral cytotoxic lymphocytes and natural killer cells, with upregulation of immunoinhibitory receptors, is believed to contribute to SARS-CoV-2 viral persistence. (Ramakrishnan RK, et al. Unraveling the Mystery Surrounding Post-Acute Sequelae of COVID-19. Front Immunol. 2021 Jun 30;12:686029)
- In chronic COVID-19, natural killer cells and T cells become exhausted and their decreased numbers leads to lymphopaenia/lymphocytopaenia (abnormally low counts of lymphocytes). This occurs with CD8+ T cells but sometimes also B cells. (Fathi N, Rezaei N. Lymphopenia in COVID-19: Therapeutic opportunities. Cell Biol Int. 2020 Sep;44(9):1792-1797; Tavakolpour S, et al. Lymphopenia during the COVID-19 infection: What it shows and what can be learned. Immunol Lett. 2020;225: 31–32)

Persistent viral fragments triggering inflammation

- There is evidence that **fragments of the virus can persist for months, where they might promote inflammation**. **Viral fragments may or may not persist in viral reservoirs. The fragments are normally proteins, not the RNA needed for viral reactivation,** Even months after an infection, mRNA from SARS-CoV-2, as well as viral proteins, have been detected in the **intestines** of infected individuals. (Marx V. Scientists set out to connect the dots on long COVID. Nature methods, 2021; 18(5), 449–453)
- Histopathological studies have found evidence of SARS-CoV-2 viral particles in endothelial cells of the **kidneys and lungs**. (Gupta, A., et al. Extrapulmonary manifestations of COVID-19. Nat Med, 2020, 26, 1017–1032)
- The levels of both intermediate (CD14+, CD16+) and non-classical monocytes (CD14Lo, CD16+) were significantly elevated in Long COVID patients and a statistically significant number of non-classical monocytes containing **SARS-CoV-2 spike (S1) protein were found in Long COVID patients up to 15 months post-infection**. Fragmented SARS-CoV-2 RNA was also found in Long COVID patients. (Patterson BK, et al. Persistence of SARS CoV-2 S1 Protein in CD16+ Monocytes in Post-Acute Sequelae of COVID-19 (PASC) up to 15 Months Post-Infection. Front Immunol. 2022;12:746021)
- Viral elements have been found within endothelial cells and an accumulation of inflammatory cells, with evidence of endothelial and inflammatory cell death. This suggests that SARS-CoV-2 infection facilitates the induction of endotheliitis in several organs as a direct consequence of viral involvement and of the host inflammatory response. In addition, induction of apoptosis and pyroptosis might have an important role in endothelial cell injury in patients with COVID-19. COVID-19-endotheliitis could explain the systemic impaired microcirculatory function in different vascular beds and their clinical sequelae in patients with COVID-19. (Varga Z, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020 May 2;395(10234):1417-1418)
- Viral proteins have been found in the brainstem and cranial nerves of 53% of patients in autopsies. Neuroinvasion can trigger neuroinflammation, thereby altering CNS function and producing Long Covid symptoms. (Matschke J, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. The Lancet Neurology 2020. doi: 10.1016/s1474-4422(20)30308-2; Low R et al. A Cytokine-based model for the pathophysiology of Long COVID symptoms. 2020; 10.31219/osf.io/7gcnv)
- Intestinal biopsies obtained from asymptomatic individuals at 4 months after the onset of coronavirus disease 2019 COVID-19) revealed the persistence of SARS-CoV-2 nucleic acids and immunoreactivity in the small bowel of 50% of individuals. (Gaebler C, et al. Evolution of antibody immunity to SARS-CoV-2. Nature. 2021; 591, 639–644)

Antibody-dependent enhancement (ADE)

- **Antibody-dependent enhancement (ADE) occurs when antibodies target a virus without neutralising it. The antibody can then facilitate endocytosis of the virus (i.e. taking it into the cell) and enhance viral replication, immune activation and massive inflammatory responses.**
- ADE promotes the virus to be recognised by the target cell, facilitates entry to the target cell and also affects the signal transmission in the target cell. It has been noted in Japanese encephalitis virus, West Nile virus, Murray Valley virus, dengue virus, Zika virus, Ebola virus, human immunodeficiency virus (HIV), Coxsackie B virus and respiratory syndrome virus.
- ADE has been proposed as a possible mechanism of both severe COVID-19 and Long COVID. It induced enhanced immune activation and is commonly seen with pathogens that cause respiratory infections.
- Circulating antigen-antibody complexes can further aggravate the cytokine storm leading to increased levels of several cytokines and increased biomarkers such as CRP and D dimer.

(Xu L, et al. Antibody dependent enhancement: Unavoidable problems in vaccine development. Advances in immunology, 2021; 151, 99–133; Brodin, P. Immune determinants of COVID-19 disease presentation and severity. Nat Med, 2021; 27, 28–33; Garg S, et al. Unraveling the mystery of Covid-19 cytokine storm: From skin to organ systems. Dermatol Ther. 2020;33(6):e13859; Sánchez-Zuno GA, et al. A review: Antibody-dependent enhancement in COVID-19: The not so friendly side of antibodies. Int J Immunopathol Pharmacol. 2021 Jan-Dec;35:20587384211050199)

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Tissue damage from acute COVID-19

- SARS-CoV-2 virus binds to ACE2 receptors present throughout the body and can adversely affect virtually every system of the body. Different organs may be affected in different patients, in a temporal course unrelated to viral load.
- It has long been known that **COVID-19 causes damage to the bodies' major organs, including heart, kidney, lung, liver and others**. The S1 spike protein, a glycoprotein, readily crosses the blood–brain barrier in mice and is taken up by brain regions and can enter the parenchymal brain space through the ACE receptor.
- This damage will not disappear as soon as the patient is free of the virus; it will persist, at least for a few months.
- The British Society for Immunology suggests that damage caused by the immune system's response to the virus, rather than the virus itself, may be the cause of Long COVID symptoms.

(Yan Z, et al. Long COVID-19 Syndrome: A Comprehensive Review of Its Effect on Various Organ Systems and Recommendation on Rehabilitation Plans. Biomedicines. 2021 Aug 5;9(8):966; Rhea EM, et al. The S1 protein of SARS-CoV-2 crosses the blood–brain barrier in mice. Nat Neurosci, 2021, 24, 368–378; Jain U. Effect of COVID-19 on the Organs. Cureus. 2020;12(8):e9540; https://www.mayoclinic.org/diseases-conditions/coronavirus/in-depth/coronavirus-long-term-effects/art-20490351; https://www.immunology.org/coronavirus/immunology-and-covid-19/report-long-term-immunological-health-consequences-covid-19; Silva Andrade B, et al. Long-COVID and Post-COVID Health Complications: An Up-to-Date Review on Clinical Conditions and Their Possible Molecular Mechanisms. Viruses. 2021 Apr 18;13(4):700)

Persistent excess inflammation

- **Persistent excess inflammation can be a result of an over-active immune system or an exhausted immune system (lymphopaenia).** It can be present after host clearance of the SARS-CoV-2 infection.
- Natural killer cells and T cells can become exhausted and their decreased numbers leads to lymphopaenia or lymphocytopaenia (abnormally low counts of lymphocytes); this occurs with CD8+ T cells but sometimes also B cells. **Lymphopaenia causes immune activation and release of the excess inflammatory cytokines in an attempt to compensate.** (Fathi N, Rezaei N. Lymphopenia in COVID-19: Therapeutic opportunities. Cell Biol Int. 2020 Sep;44(9):1792-1797; Tavakolpour S, et al. Lymphopenia during the COVID-19 infection: What it shows and what can be learned. Immunol Lett. 2020;225: 31–32)
- This condition has been observed in some individuals infected with SARS-CoV-2, especially those who were admitted to intensive care units. T cells may exhibit functional exhaustion or an activated profile in the presence of cytotoxic CD8+ T cells. (Tiyo, B. T., et al. (2021). What Happens to the Immune System after Vaccination or Recovery from COVID-19?. Life (Basel, Switzerland), 11(11), 1152)
- An Australian study observed that the long COVID group had highly activated innate immune cells but lacked naive T and B cells, even in those with only mild acute infection. They also noted that after 8 months, type I IFN (IFN-β) and type III IFN (IFN-λ1) interferons (a protein that body cells make in response to the presence of viruses), instead of disappearing as the infection cleared, remained at a high level compared to those without Long COVID. The team believed that these elevated interferons could lead to fatigue, depression, headache and other long COVID symptoms. (Phetsouphanh, C., et al. Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection. Nat Immunol 23, 210–216 (2022))

(Datta SD, et al. A Proposed Framework and Timeline of the Spectrum of Disease Due to SARS-CoV-2 Infection: Illness Beyond Acute Infection and Public Health Implications. JAMA. 2020;324(22):2251–2252; https://www.the-scientist.com/features/mechanisms-of-long-covid-remain-unknown-but-data-are-rolling-in-69066)

Overactivation of the renin-angiotensin system (RAS)

- The renin-angiotensin system (RAS) plays a key role in maintaining the physiological balance of the body, thereby influencing the function of multiple organ systems. ACE2 catalyses the conversion of angiotensin II (Ang II) to angiotensin.
- **When the virus binds to the ACE2 receptor, it leads to a decreased density of the receptor on the vascular tissue and accumulation of Ang II, which can lead to overactivation of the RAS** and causes vasoconstriction, profibrotic, and proinflammatory effects, as well as tissue fibrosis.
- Elevated plasma Ang II levels have been associated with lung injury and pathogenesis of critically ill COVID-19 patients. The imbalance between ACE2/angiotensin axis and RAS have also been implicated in **multi-organ injury** associated with COVID-19. Dysregulation of the RAS may also create a persistent cardiometabolic demand in recovered patients.
- In COVID-19 patients, 81% (and 93% who were hospitalised) had detectable post-infection ACE2 antibodies, suggesting that these antibodies decrease ACE2 activity, which could lead to an increase in the abundance of Ang II, which causes a proinflammatory state.

(Ramakrishnan RK, et al. Unraveling the Mystery Surrounding Post-Acute Sequelae of COVID-19. Front Immunol. 2021 Jun 30;12:686029; Silva Andrade B, et al. Long-COVID and Post-COVID Health Complications: An Up-to-Date Review on Clinical Conditions and Their Possible Molecular Mechanisms. Viruses. 2021 Apr 18;13(4):700; Arthur JM, et al. Development of ACE2 autoantibodies after SARS-CoV-2 infection. PLoS One. 2021; 16(9): e0257016)

Endothelial dysfunction

- COVID-19, particularly in the later complicated stages, is an **endothelial disease and can cause systemic endotheliitis**, likely due to the high expression of endothelial cells for the ACE2 receptor. (Libby P, Lüscher T. COVID-19 is, in the end, an endothelial disease. Eur Heart J. 2020 Sep 1;41(32):3038-3044; Seitz A, Ong P. Endothelial dysfunction in COVID-19: A potential predictor of long-COVID? Int J Cardiol. 2022 Feb 15;349:155-156)
- Long COVID may be due in part to **vascular endothelium injury and dysfunction**. A study showed that up to 6 months after diagnosis, fatigue, chest pain and neuro-cognitive difficulties were significantly associated with persistent endothelium dysfunction. (Charfeddine S, et al. Long COVID 19 Syndrome: Is It Related to Microcirculation and Endothelial Dysfunction? Insights From TUN-EndCOV Study. Front Cardiovasc Med. 2021 Nov 30;8:745758)
- Elevated levels of circulating endothelial cells (CECs) were found in COVID-19 convalescent patients, which correlated with several proinflammatory and activated T lymphocyte-associated cytokines, suggesting cytokine-driven endothelial dysfunction. (Chioh FW, et al. Convalescent COVID-19 patients are susceptible to endothelial dysfunction due to persistent immune activation. Elife. 2021 Mar 23;10:e64909)
- Direct viral invasion via ACE2 receptor was seen in cardiac tissue (pericytes, endothelial cells, cardiomyocytes, cardiofibroblasts, epicardial adipose cells and vascular cells), with hyperinflammation and endothelial dysfunction affecting the integrity of the myocardium and pericardium, which may perpetuate cardiovascular damage in COVID-19 survivors. (Ramakrishnan RK, et al. Unraveling the Mystery Surrounding Post-Acute Sequelae of COVID-19. Front Immunol. 2021 Jun 30;12:686029)
- Infection-mediated endothelial injury and endotheliitis were found in multiple vascular beds (including the lungs, kidney, heart, small intestine and liver) in patients with COVID-19. (Gupta, A., et al. Extrapulmonary manifestations of COVID-19. Nat Med, 2020, 26, 1017–1032)
- At 6 months after infection, c10% of recovered patients had persistent symptoms as well as high levels of endothelial activation markers. (Ong SWX, et al. Persistent Symptoms and Association With Inflammatory Cytokine Signatures in Recovered Coronavirus Disease 2019 Patients. Open Forum Infect Dis. 2021 Apr 2;8(6):ofab156)

Vascular injury

- SARS-CoV-2-mediated endothelial damage induces systemic vascular disease and a growing body of evidence now suggests that this **macro- and microvascular dysfunction and injury contributes to long COVID**. (Seitz A, Ong P. Endothelial dysfunction in COVID-19: A potential predictor of long-COVID? Int J Cardiol. 2022 Feb 15;349:155-156)
- Elevated levels of circulating endothelial cells (CECs), a biomarker of vascular injury, were found in COVID-19 convalescent patients. (Chioh FW, et al. Convalescent COVID-19 patients are susceptible to endothelial dysfunction due to persistent immune activation. Elife. 2021 Mar 23;10:e64909)
- Microvascular injury is a suggested cause of Long COVID (Ramakrishnan RK, et al. Unraveling the Mystery Surrounding Post-Acute Sequelae of COVID-19. Front Immunol. 2021 Jun 30;12:686029).

Hypercoagulation

- Infection-mediated **endothelial injury and endotheliitis** were found in multiple vascular beds in COVID-19 patients. Here they **can trigger excessive thrombin production,** inhibit fibrinolysis, and activate complement pathways, **initiating thrombo-inflammation and ultimately leading to microthrombi deposition.**
- Platelet–neutrophil cross-communication and activation of macrophages in this setting can facilitate a variety of proinflammatory effects, such as cytokine release, the formation of neutrophil extracellular traps (NETs, and fibrin and/or microthrombus formation). NETs further damage the endothelium and activate both extrinsic and intrinsic coagulation pathways.
- Additionally, direct COVID-mediated effects may also lead to an imbalance of pro- and anti-coagulant pathways, with the presence of fibrinous exudates and microthrombi in histopathological examinations. The widespread presence of ACE-2 receptors in the heart and lungs increase the risk of SARS-CoV-2-related endothelial injury and thrombo-inflammation.
- Furthermore, disproportionate activation of the complement system can lead to hypercoagulation and thrombotic complications, pulmonary embolism, cardiac injury and stroke.
- The increased stimulation of inflammatory cytokines IL-1 and IL-6 and the excessive activity of Ang II bring endothelial activation, increased permeability, and co-expression of adhesion molecules, thus generating a prothrombotic phenotype. This generates haemostatic changes that leave the endothelium inflamed, pre-adhesive and prothrombotic; the ongoing tissue damage causes endotheliitis.
- Inflammation in the vascular system can result in diffuse microangiopathy with thrombosis.

(https://www.immunology.org/coronavirus/immunology-and-covid-19/report-long-term-immunological-health-consequences-covid-19; Gupta, A., et al. Extrapulmonary manifestations of COVID-19. Nat Med, 2020, 26, 1017–1032; Silva Andrade B, et al. Long-COVID and Post-COVID Health Complications: An Up-to-Date Review on Clinical Conditions and Their Possible Molecular Mechanisms. Viruses. 2021 Apr 18;13(4):700; Liu PP, et al. The Science Underlying COVID-19: Implications for the Cardiovascular System. Circulation. 2020 Jul 7;142(1):68-78)

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Induction of autoimmune conditions through molecular mimicry

- The **Autoimmune Registry** has determined that biomarkers of immune system activity similar to those seen in many autoimmune and autoinflammatory diseases justify the **inclusion of Long COVID on its list of autoimmune conditions** (https://www.autoimmuneregistry.org/long-covid-announcement). They cite symptom similarity which they believe is not coincidental: Fatigue; An unpredictable flare-remission pattern of symptoms; "Brain fog", an interruption of memory, attention, and thought processing.
- They also point out that myocarditis and blood clots similar to those found in the autoimmune disease antiphospholipid syndrome have been reported in patients with COVID-19 and Long COVID, while high levels of antibodies to type I interferons (IFNs) have been associated with severe COVID-19.
- Recent findings suggest the ability of **SARS-CoV-2-specific antibodies and autoreactive T cells to cross-react with host proteins**: cytokines, chemokines, complement proteins, immunomodulatory proteins, metalloproteinases endothelial cell surface proteins and others. SARS-CoV-2-neutralising antibodies and autoantibodies have been detected and may cross-react with self-antigens; antinuclear antibodies were also present. Studies found that 52% of COVID-19 patients had antiphospholipid autoantibodies and 69% of ICU patients tested positive for any kind of autoantibody related to autoimmune rheumatic diseases. (Ramakrishnan RK, et al. Unraveling the Mystery Surrounding Post-Acute Sequelae of COVID-19. Front Immunol. 2021 Jun 30;12:686029)
- A review highlights studies development of autoimmune conditions in COVID patients: Guillain-Barré syndrome, Miller Fisher Syndrome, antiphospholipid syndrome, immune thrombocytopaenia purpura, systemic lupus erythematosus (SLE) and Kawasaki disease. (Halpert G, Shoenfeld Y. SARS-CoV-2, the autoimmune virus. Autoimmun Rev. 2020 Dec;19(12):102695)

Mitochondrial dysfunction

- **Mitochondrial function in COVID-19 infected patients is impaired, and these patients cannot produce their required energy by this pathway, leading to increased glycolysis to compensate.** Mitochondrial dysfunction and metabolic alterations with an increase in glycolysis in peripheral blood mononuclear cells were demonstrated in patients with COVID-19. (Ajaz S, et al. Mitochondrial metabolic manipulation by SARS-CoV-2 in peripheral blood mononuclear cells of patients with COVID-19. Am J Phys Cell Phys. 2021;320(1):C57–65)
- **PET scans have shown brain hypometabolism in Long COVID-patients** (Guedj E, et al. 18F-FDG brain PET hypometabolism in post-SARS-CoV-2 infection: substrate for persistent/delayed disorders? Eur J Nucl Med Mol Imaging. 2021 Feb;48(2):592-595; Sollini M, et al. Long COVID hallmarks on [18F]FDG-PET/CT: a case-control study. Eur J Nucl Med Mol Imaging. 2021 Sep;48(10):3187-3197)
- SARS-CoV-2 infection may functionally divert the bioenergetic capacity of infected cells to support viral replication. It is possible that mitochondrial targeting by the virus may be the substrate for the emergence of cognitive impairment or ‘brain fog’. Neurons have a particularly high oxygen demand, so any hypoxia will also be detrimental. (Stefano GB. Historical Insight into Infections and Disorders Associated with Neurological and Psychiatric Sequelae Similar to Long COVID. Med Sci Monit. 2021 Feb 26;27:e931447; Stefano GB, et al. Selective Neuronal Mitochondrial Targeting in SARS-CoV-2 Infection Affects Cognitive Processes to Induce 'Brain Fog' and Results in Behavioral Changes that Favor Viral Survival. Med Sci Monit. 2021 Jan 25;27:e930886)
- 40 days post-infection, a study revealed that six proteins were significantly altered, two of which originated from the mitochondria, peroxiredoxin 3 (PRDX3), an antioxidant, and carbamoyl phosphate synthase (CPS1). The increase of PRDX3 in serum of COVID-19 patients is likely indicative of a continued mitochondrial stress response. CPS1 is a major mitochondrial urea cycle enzyme in hepatocytes; serum CPS1 originates from the bile duct and is usually rapidly cleared by peripheral blood mononuclear cells but levels may be reduced due to increased circulation and activity of peripheral blood mononuclear cells. (Doykov, I., et al (2020). 'The long tail of Covid-19' - The detection of a prolonged inflammatory response after a SARS-CoV-2 infection in asymptomatic and mildly affected patients. F1000Research, 9, 1349)

Reactivation of other dormant viruses

- A 2021 study found that **67% of Long COVID patients**, versus 10% of control subjects, **were positive for Epstein Barr virus (EBV) reactivation** based on IgG or IgM antibodies. The study showed that reactivation may occur soon after or concurrently with COVID-19 infection. **The researchers suggest that many long COVID symptoms may not be a direct result of the SARS-CoV-2 virus but may be the result of COVID-19-induced EBV reactivation**. (Gold JE; et al. Investigation of Long COVID Prevalence and Its Relationship to Epstein-Barr Virus Reactivation. Pathogens 2021, 10, 763)
- **In fact, EBV also increases susceptibility of epithelial cells to infection by SARS-CoV-2.** (Verma D, et al. Epstein-Barr Virus Lytic Replication Induces ACE2 Expression and Enhances SARS-CoV-2 Pseudotyped Virus Entry in Epithelial Cells. J Virol. 2021 Jun 10;95(13):e0019221)
- At least 65% of the UK population harbours the Epstein Barr virus (EBV), a member of the herpes family of viruses, contracted in childhood. For most people EBV is harmless and symptomless, but if it is reactivated, it can cause serious symptoms; EBV has long been associated with the onset of CFS/ME. (Winter, J.R., et al. Predictors of Epstein-Barr virus serostatus in young people in England. BMC Infect Dis, 2019, 19, 1007)
- Patients with protracted sepsis had markedly increased frequency of detectable viral DNA for: cytomegalovirus (CMV) (24%), Epstein-Barr (EBV) (53%), herpes simplex (HSV) (14%), human herpes virus-6 (10%), and the anellovirus TTV (77.5%). 43% of sepsis patients had presence of two or more viruses. Reactivation of latent viruses is common with prolonged sepsis, with frequencies similar to those occurring in transplant patients on immunosuppressive therapy and consistent with development of an immunosuppressive state. (Walton AH, et al. Reactivation of multiple viruses in patients with sepsis. PLoS One. 2014 Jun 11;9(2):e98819)
- These viruses generally persist in dormant, latent or non-cytolytic forms, but may reactivate under conditions of stress or immunosuppression. Indeed, people regarded as healthy have been shown to harbour a wide range of persistent viruses that are capable of activation under such conditions. (Proal AD & VanElzakker MB. Long COVID or Post-acute Sequelae of COVID-19 (PASC): An Overview of Biological Factors That May Contribute to Persistent Symptoms. Frontiers in microbiology. 2021; 12, 698169)

Microbiome alteration

- SARS-CoV-2 can cause gastrointestinal symptoms during the early phases of the disease and this intestinal dysfunction induces changes in the microbiome which persist. These can activate immune cells and increase inflammatory cytokines leading to systemic inflammation as well as longer term gut dysbiosis. (Villapol S. Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. Transl Res. 2020 Dec;226:57-69; Silva Andrade, B., et al. (2021). Long-COVID and Post-COVID Health Complications: An Up-to-Date Review on Clinical Conditions and Their Possible Molecular Mechanisms. Viruses, 13(4), 700)
- **In Long COVID patients, microbiota status was not restored to normal levels 6 months post-COVID-19.** (Zuo T, et al. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. Gastroenterology. 2020;159(3):944–55 e8; Chen Y, et al. Six-month follow-up of gut microbiota richness in patients with COVID-19. Gut. 2022 Jan;71(1):222-225)
- Gut microbiota composition at admission was associated with occurrence of Long COVID. **The gut microbiome profile of Long COVID patients** were characterised by higher levels of *Ruminococcus gnavus*, *Bacteroides vulgatus* and lower levels of *Faecalibacterium prausnitzii*. Persistent respiratory symptoms were correlated with opportunistic gut pathogens, while neuropsychiatric symptoms and fatigue were correlated with nosocomial gut pathogens, including *Clostridium innocuum* and *Actinomyces naeslundii*. Butyrate-producing bacteria, including *Bifidobacterium pseudocatenulatum* and *Faecalibacterium prausnitzii* showed the largest inverse correlations with Long COVID at 6 months. (Liu Q, et al. Gut microbiota dynamics in a prospective cohort of patients with post-acute COVID-19 syndrome. Gut. 2022 Mar;71(3):544-552)

Demyelination in brain and nervous system

- **We know that demyelination is a cause of multiple sclerosis (also an autoimmune condition) but can also cause other neurological conditions.**
- **A systematic review of 60 studies found that COVID-19** (in common with other viruses) **was associated with CNS demyelination** (removal of the protective myelin sheath coating nerves), predisposing patients to demyelinating conditions such as multiple sclerosis. The precise mechanism is not clear but may involve the inflammatory response activating myelin-specific T cells or production of anti-myelin antibodies triggered by the virus. (Ismail II, Salama S. Association of CNS demyelination and COVID-19 infection: an updated systematic review. J Neurol. 2022 Feb;269(2):541-576)
- COVID-19 infection is a risk factor for demyelination both in the peripheral and central nervous systems. (Shabani Z. Demyelination as a result of an immune response in patients with COVID-19. Acta Neurol Belg. 2021 Aug;121(4):859-866)
- Brain MRI scans indicated new demyelination injuries, possibly due to delayed immune response. (Zanin L, et al, SARS-CoV-2 can induce brain and spine demyelinating lesions. Acta Neurochirurgica, 2020:1–4)

Mechanisms of Long COVID – the medical hypotheses

Effect of SARS-CoV-2 itself

- The virus is still harboured in a reservoir organ which is sheltered from the immune system.
- SARS-CoV-2 is a superantigen
- Delayed viral clearance due to immune exhaustion.
- Persistent viral fragments, though not infectious, may still be triggering inflammation.
- The virus has been reverse-transcribed and integrated into our DNA
- Antibody dependent enhancement (ADE)

Direct after-effects of SARS-CoV-2

- Tissue damage from acute COVID-19.
- Persistent excess inflammation
- Overactivation of the renin-angiotensin system (RAS)
- Endothelial dysfunction, vascular injury and hypercoagulation

Indirect effects of SARS-CoV-2

- Induction of autoimmune conditions through molecular mimicry
- Mitochondrial dysfunction
- Reactivation of other dormant viruses
- Microbiome alteration
- Demyelination in brain and nervous system

Contributory factors

- Genetics
- Diet and malnutrition
- Lack of exercise and physical deconditioning due to immobility while ill
- Poor methylation/high homocysteine

Contributory factors: Genetics

- The reason that some individuals are more prone to develop long COVID possibly lies in their genetic profile primarily related to the immune system, such as human leukocyte antigen (HLA). (Akbarialiabad, https://link.springer.com/article/10.1007%2Fs15010-021-01666-x)
- Some data shows that remission of symptoms can be related to genetic variations. A study revealed that the rs1173773 variant on chromosome 5 is strongly associated with remission of symptoms. Patients carrying rs1173773 Allele C had a greater remission of symptoms compared to Allele T carriers: 59%, 54%, and 44% of individuals with CC, CT, TT genotypes, respectively, had remission of symptoms. The results are novel and are not replicated yet. (Dubé MP, et al. Genetics of symptom remission in outpatients with COVID-19. Sci Rep 11, 2021; 10847)
- A genome-wide association study in 2000 Japanese COVID-19 patients found that many COVID-related genes except DOCK2 overlapped with European populations. Notably, the DOCK2 variant that increased COVID-19 risk was relatively common in East Asians including Japan but very rare in Europeans. The research on host genomics has yielded promising results for personalised medicine, but its application to clinical practice is still in the embryonic stage. (Namkoon, https://europepmc.org/article/PPR/PPR341291)

Contributory factors: Diet and malnutrition

- Diet and lifestyle factors might contribute Long COVID, as well as prevent an ability to recover from symptoms. Malnutrition from being unwell and living alone or following a prolonged hospital stay can contribute to long-COVID symptoms.
- **A meta-analysis showed that Long COVID patients had a high risk of malnutrition** (Abate SM, et al. (2021) Prevalence and outcomes of malnutrition among hospitalized COVID-19 patients: A systematic review and meta-analysis. Clin Nutr ESPEN. 43:174-183).
- This can be exacerbated by alterations in taste and smell, well-reported in some people after infection, which can contribute to low appetite and inadequate dietary intake. As a result, they may not get enough micronutrients or macronutrients that are essential to a normal function of body including immune system.
- Other factors such as impaired mental health can influence poor dietary consumption and malnutrition.

Contributory factors: Lack of exercise and physical deconditioning due to immobility while ill

- Restrictive measures during lockdowns, isolation or quarantine may have affected physical activity. Not getting enough physical exercise can contribute to poor health, including the accumulation of body fat, muscle maintenance, increased mental distress, immune dysfunction and increased inflammatory effect. (Dimitrov S., et al. Inflammation and exercise: Inhibition of monocytic intracellular TNF production by acute exercise via β2-adrenergic activation. Brain Behav Immun. 2017l 61:60-68; da Silveira MP, et al. Physical exercise as a tool to help the immune system against COVID-19: an integrative review of the current literature. Clin Exp Med; 2021, 21: 1–14)
- Evidence suggests that there was a significant reduction in physical activities during the lockdown in both adults and children (Stockwell, S., et al. Changes in physical activity and sedentary behaviours from before to during the COVID-19 pandemic lockdown: a systematic review. BMJ open sport & exercise medicine, 2021; 7(1), e000960; Okely, AD. et al. Global effect of COVID-19 pandemic on physical activity, sedentary behaviour and sleep among 3- to 5-year-old children: a longitudinal study of 14 countries. BMC Public Health 2021; 21, 940).
- Sedentary activities such as sitting and watching/using screens increased during this period. Insufficient physical activity is strongly associated with severe COVID symptoms, ICU admission, deaths as well as other commodities such as obesity, cardiovascular diseases, and hypertension. Physical activity of individuals with lingering COVID symptoms was influenced up to 6 months post infection. (Sallis, R. et al. Physical inactivity is associated with a higher risk for severe COVID-19 outcomes: A study in 48 440 adult patients. Br. J. Sports Med. 2021)
- Physical deconditioning while hospitalised is the main mechanism of impaired exercise response in COVID-19 survivors (Rinaldo RF, et al. Deconditioning as main mechanism of impaired exercise response in COVID-19 survivors. Eur Respir J. 2021;58(2):2100870. Published 2021 Aug 26. doi:10.1183/13993003.00870-2021)

Contributory factors: Poor methylation and high homocysteine

- There is a correspondence between symptoms described by patients with Long COVID and **pernicious anaemia** (PA). PA is an autoimmune disease caused by deficient synthesis of gastric intrinsic factor and subsequent malabsorption of vitamin B12; moreover, ME/CFS can also be a B12-responsive syndrome.
- **SARS-CoV-2 seems to induce an increased demand for methyl groups whilst simultaneously impairing their supply** due to virus-mediated oxidative stress, which causes changes in the B12-dependent methionine synthase (MS) reaction, which allows the conversion of homocysteine back to methionine. (McCaddon A, Regland B. COVID-19: A methyl-group assault? Med Hypotheses. 2021 Apr;149:110543)
- SARS-CoV-2 may destroy one-carbon metabolism as it undergoes rapid replication during the viraemia stage, when it will hijack the hosts cells' supply of methyl groups and will increase oxidative stress due to high homocysteine, as well as depleting serine and glutathione. High homocysteine has been suggested as a potential predictive biomarker for COVID-19 infection severity and outcome. (Hayden MR & Tyagi SC. Impaired Folate-Mediated One-Carbon Metabolism in Type 2 Diabetes, Late-Onset Alzheimer's Disease and Long COVID. Medicina, 2021; 58(1), 16)

Recap on viral reservoirs in Long COVID

- Viral reservoirs hide virus-infected cells which remain hidden from the immune system.
- There are SARS-CoV-2 reservoirs in Long COVID patients in multiple organs including the brain, lung, myocardium, lymphoid tissue (gut, lymph nodes and spleen) but also in the central nervous system.
- Where the immune system is dysfunctional or distracted, the infection can be reactivated from viral reservoirs.
- There are several reports of relapse or reactivation of SARS-CoV-2 in recovered COVID-19 patients.

Are long COVID sufferers still PCR-positive?

- **People who have long COVID are not considered infectious and most studies that tested for recurrent COVID showed that long COVID patients were PCR negative.**
- **But evidence for persistent infection is shown by prolonged viral shedding in faeces but will not be picked up in the routine testing of the upper respiratory tract**. Persistent antibodies, particularly IgG, however, have been observed to coexist with viral persistence, suggesting that the antibodies are non-neutralising.
- But one study showed that 25% of Long COVID patients were still PCR positive after 2 months and another found that some recovered patients tested positive but there was no difference in Long COVID symptom prevalence between those testing positive or negative.
- A growing number of studies show that some patients infected with SARS-CoV-2 do not successfully clear the virus over long periods of time; individuals who were still PCR-positive after recovery displayed SARS-CoV-2-specific CD8 T-cell responses of significantly increased breadth and magnitude, leading the team to suggest that such subjects might still harbour replicating virus.

(Salmon-Ceron D, et al. Clinical, virological and imaging profile in patients with prolonged forms of COVID-19: A cross-sectional study. J Infect. 2021 Feb;82(2):e1-e4; Cevik M, et al. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. Lancet Microbe. 2021 Jan;2(1):e13-e22; Plebani M. Persistent viral RNA shedding in COVID-19: Caution, not fear. EBioMedicine. 2021 Feb;64:103234; Proal AD & VanElzakker MB. Long COVID or Post-acute Sequelae of COVID-19 (PASC): An Overview of Biological Factors That May Contribute to Persistent Symptoms. Frontiers in microbiology. 2021; 12, 698169; Wang B, et al. Long-term coexistence of SARS-CoV-2 with antibody response in COVID-19 patients. J Med Virol 92; 2020: 1684–1689)

Viral reservoirs: Reactivation

- **A meta-analysis estimated that c12% of recovered patients tested positive for SARS-CoV-2 reactivation** (Mattiuzzi C, et al. SARS-CoV-2 recurrent RNA positivity after recovering from coronavirus disease 2019 (COVID-19): a meta-analysis. Acta Biomed. 2020 Sep 7;91(3):e2020014).
- **A number of studies have shown that reactivation of dormant viruses may contribute to severe COVID-19.**
- **Reactivation of SARS-CoV-2, or any other virus, can only come about when the immune system is dysfunctional or in some way suppressed. This can occur either through poor maintenance (inadequate levels of vitamin D etc), though the induction of CARS (compensatory anti-inflammatory response syndrome) or immune exhaustion.**
- It is important to distinguish between reinfection and reactivation. Early in the pandemic, reactivation cases were wrongly categorised as cases of reinfection. (Sciscent BY et al. COVID-19 reinfection: the role of natural immunity, vaccines, and variants. Journal of community hospital internal medicine perspectives. 2021; 11(6), 733–739; (Jacobs JJL. Persistent SARS-2 infections contribute to long COVID-19. Med Hypotheses. 2021; 149: 110538)
- CARS is an immunologic phenomenon that was increasingly noticed to occur in sepsis. Its precursor, the systemic inflammatory response syndrome (SIRS) was tasked with killing infectious organisms through activation of the immune system, while CARS is a counter-regulatory systemic deactivation of the immune system tasked with restoring homeostasis from an inflammatory state; it has a distinct set of cytokines and cellular responses. Patients with CARS are at a higher risk for reactivation of SARS-CoV-2 or any other latent virus, as well as infection by opportunistic pathogens. (Ward NS et al. The compensatory anti-inflammatory response syndrome (CARS) in critically ill patients. Clinics in chest medicine. 2008; 29(4), 617–viii; Oronsky B, et al. A Review of Persistent Post-COVID Syndrome (PPCS). Clin Rev Allergy Immunol. 2021 Feb 20:1–9)

Viral reservoir protection: biofilms

- It was not until 2010 that researchers discovered that **viruses can build and hide in biofilms in a similar manner to bacteria, in order to evade the immune system** and improve the chance of survival. Biofilms are thought to form in up to 80% of human infections. (Von Borowski RG, Trentin DS. Biofilms and Coronavirus Reservoirs: a Perspective Review. Appl Environ Microbiol. 2021 Aug 26;87(18):e0085921)
- Using the human T-cell leukaemia virus type 1 (HTLV-1), a retrovirus, scientists showed that the virus formed protective and adhesive 'biofilm-like' structures on the surface of infected cells. These biofilms are aggregates of viruses embedded in a **carbohydrate-rich cocoon structure known as the matrix (comprising DNA, protein and polysaccharides)**.
- In the biofilm, HTLV-1 is far more easily transmitted than in its free, isolated state, as the biofilm itself efficiently transfers viruses between cells. **By removing the viral biofilm from the surface of the infected cells, researchers achieved an 80% reduction in reactivation rates.** (Pais-Correia AM et al. Biofilm-like extracellular viral assemblies mediate HTLV-1 cell-to-cell transmission at virological synapses. Nature Medicine, 2010; 16 (1): 83)
- One of the important differences between bacterial and viral biofilm is that the matrix of normal bacterial biofilm formation is produced by the bacterium itself, while the matrix of viral biofilm is produced by the infected cell but is controlled by the virus. (Besharati S, Investigation of the hypothesis of biofilm formation in coronavirus (COVID-19))

Biofilm removal

- It may be tempting to try to remove the biofilm to allow the immune system to deal with viral reservoirs. I would suggest not doing so unless you know that the immune system is robust and well supported, otherwise you could do more harm than good!
- If you do want to remove the viral biofilm, there does not appear to be any specific protocol for removing it, so it is worth trying a protocol for removing bacterial biofilm:
  - Biofilm-busting enzymes (take away from food): Klaire Labs Interfase Plus; Kirkman Biofilm Defense; other enzymes such as nattokinase or serrapeptase
  - EDTA (because cells use toxic metals to bind the biofilm)
  - N-acetyl cysteine
  - Cinnamon bark oil
  - Lactoferrin

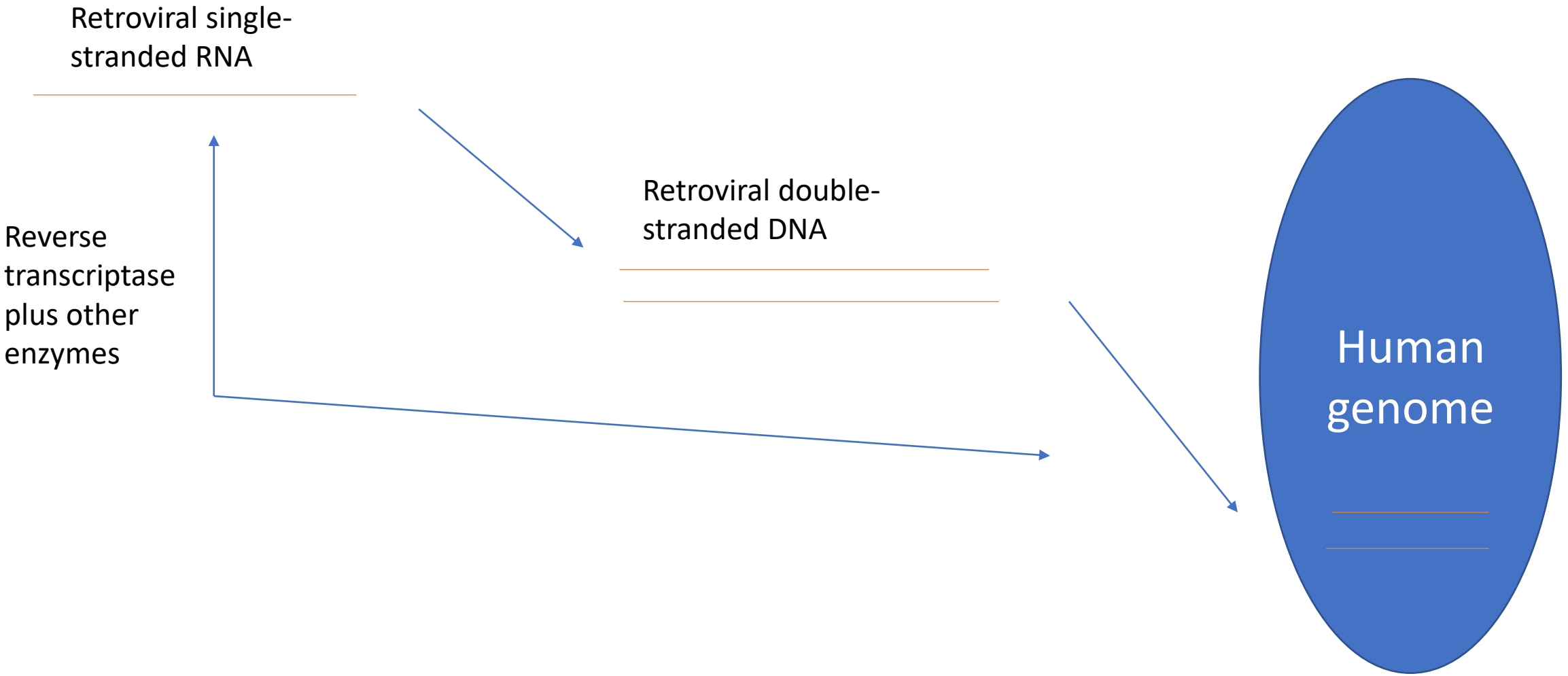
Viral reservoirs in HIV and other viruses

- Most of the viral reservoir research has been carried out on HIV.
- **Viral reservoirs contain ≥ 98% of all HIV-infected cells, with 83-95% in the gut**. The extent to which anti-retroviral therapy does not cure HIV infection is due to the persistence of HIV reservoirs in long-lived memory CD4 T cells present in the lymph nodes and intestinal tract epithelial cells.
- **Other viruses can also persist in the body:** long lived viral DNA has been found in cellular reservoirs in persistent **hepatitis B, Ebola and Zika viruses, Epstein Barr, other Herpes viruses and varicella zoster (chickenpox, shingles).**
- **Interestingly, these viruses all have retrovirus associations.**
- HIV reservoirs also exist within tissue macrophages, myeloid cells, brain microglial cells and haematopoietic stem cells (HSCs). Despite a lack of active viral production, latently HIV-infected subjects continue to exhibit aberrant cellular signalling and metabolic dysfunction, leading to cellular and systemic complications or comorbidities. These include genomic DNA damage; telomere attrition; mitochondrial dysfunction; premature ageing; and lymphocytic, cardiac, renal, hepatic, or pulmonary dysfunctions.

(https://www.nature.com/subjects/viral-reservoirs; Neurath MF, et al. Gut as viral reservoir: lessons from gut viromes, HIV and COVID-19 Gut 2021;70:1605-1608; Colón-Thillet, R., et al. Optimization of AAV vectors to target persistent viral reservoirs. Virol J, 2021; 18, 85; https://www.nature.com/articles/s41592-021-01145-z; Busman-Sahay K et al; Eliminating HIV reservoirs for a cure: the issue is in the tissue, Current Opinion in HIV and AIDS: July 2021 - Volume 16 - Issue 4 - p 200-208; Khanal S, et al. HIV-1 Latency and Viral Reservoirs: Existing Reversal Approaches and Potential Technologies, Targets, and Pathways Involved in HIV Latency Studies. Cells. 2021 Feb 23;10(2):475;)

Retroviruses and reverse transcriptase

- **Retroviruses can incorporate themselves into the human genome by employment of reverse transcriptase.**
- **Reverse transcription is the process of making a double stranded DNA molecule from a single stranded RNA template**. It is called reverse transcription as it acts in the opposite or reverse direction to regular transcription (creation of RNA using a DNA template to make proteins).
- Reverse transcriptase is an RNA-dependent DNA polymerase that was discovered in many retroviruses. It allows viral mRNA to create new DNA with the help of a nuclease, termed ribonuclease H (RNase H). The resultant DNA can then be integrated into our genome.
- Examples of retroviruses with reverse transcriptase include Human Immunodeficiency Virus (HIV) and Human T-Lymphotropic virus (HTLV).


Retroviral single-stranded RNA
Retroviral double-stranded DNA
Reverse transcriptase plus other enzymes
Human genome

Retroviral elements already in the human genome

- **Human endogenous retroviral elements (HERVs) are already present in the human genome and are pathogenic.** They have c8% of human chromosomal sequences, the remainder being families acquired from exogenous retroviruses via infection of germline cells. The abnormal expression of HERV usually becomes self-sustained and therefore in host cells that are the 'dormant enemies within'. (https://www.news-medical.net/news/20220124/Human-endogenous-retrovirus-type-W-envelope-protein-found-to-be-a-biomarker-of-COVID-19-severity.aspx)
- An *in vitro* study showed that the SARS-CoV-2 spike protein activates HERV envelope protein expression through transcription in peripheral blood lymphocytes and other tissues, including brain microglia, which correlated with disease severity, indicating lymphocyte exhaustion. **In COVID-19, HERV reactivation and the expression of HERV proteins can contribute to perpetuating pathogenic pathways underlying the severe and long-term pathology of COVID-19; HERVs were found expressed in COVID-19 post-mortem tissues**. (Charvet, https://www.medrxiv.org/content/10.1101/2022.01.18.21266111v2)
- These HERVS are known as transposable elements, or jumping genes, are now known to be responsible for many human diseases, although they **can be repressed by a robust innate immune system and methylation**. One particular kind of transposable element, the Line-1 retrotransposons deriving from HERVs, is implicated in an ever-expanding host of neurodegenerative conditions. A key finding in these studies is that not all COVID patients had significant HERV ENV activation; only 20 or 30 percent of them did. In heart tissue samples from COVID-19 patients, the HERV ENV was mainly found in endothelial cells from numerous small blood vessels and in the pericardial fatty tissue. Ominously, significant HERV ENV in patients was found in blood clots, nasal mucosa and also in the central nervous system, particularly in microglial cells, even when SARS-CoV-2 could not be detected in those tissues. (https://phys.org/news/2022-01-sars-cov-spike-protein-human-endogenous.html)

Selected features of retroviruses (mainly HIV)

- Have reverse transcriptase and can insert themselves into the human genome.
- Other viruses can predispose sufferers to develop retrovirus infections.
- Are immunosuppressive.
- Can disable the cellular protein tetherin.
- HIV antibodies can help neutralise other viruses.
- Anti-retroviral drugs can suppress non-retroviruses.

Viruses with retrovirus associations

- Hepatitis B is not a retrovirus but nevertheless **has reverse transcriptase** which can incorporate the virus into our DNA.
- Infection with the Herpes simplex virus (HSV) can **predispose sufferers to a retrovirus** (normally HIV).
- The envelope glycoprotein of **Ebola contains an immunosuppressive-like domain** similar to those found in oncogenic retroviruses.
- **Anti-retroviral drugs reduce the risk** of suffering from herpes simplex virus (Tenofovir) and Zika virus in the brain (Rilpivirine).
- **Ebola and Zika can disable the cellular protein tetherin**. Tetherin is an interferon-induced protein whose expression blocks the release of enveloped viral particles. Disabling tetherin suppresses the immune system and allows viral spread.
- Effective **monoclonal anti-Ebola antibodies also offer cross-protection against HIV**. A high proportion of those with HIV also had antibodies to Herpes simplex and varicella zoster viruses.
- **So the viruses found in viral reservoirs all have retroviral associations.**

(https://clinmedjournals.org/articles/ijva/international-journal-of-virology-and-aids-ijva-5-044.php?jid=ijva; Miller RH, Robinson WS. Common evolutionary origin of hepatitis B virus and retroviruses. Proc Natl Acad Sci U S A. 1986 Apr;83(8):2531-5; Andrei G, et al, The Anti–Human Immunodeficiency Virus Drug Tenofovir, a Reverse Transcriptase Inhibitor, Also Targets the Herpes Simplex Virus DNA Polymerase, The Journal of Infectious Diseases, Volume 217, Issue 5, 2018, 790–801; Volchkov VE, et al. The envelope glycoprotein of Ebola virus contains an immunosuppressive-like domain similar to oncogenic retroviruses. FEBS Lett. 1992 Jul 6;305(3):181-4; Sariyer IK, et al. Suppression of Zika Virus Infection in the Brain by the Antiretroviral Drug Rilpivirine. Mol Ther. 2019 Dec 4;27(12):2067-2079; Herrlein ML, et al. Catch me if you can - the crosstalk of ZIKV and the restriction factor Tetherin. J Virol. 2021 Dec 22:jvi0211721; https://www.ucsf-ahp.org/can-hiv-activate-herpes-varicella-zoster-virus/; Perez-Zsolt, D., et al. Anti-Siglec-1 antibodies block Ebola viral uptake and decrease cytoplasmic viral entry. Nat Microbiol 4, 1558–1570 (2019; Wallace MR, et al. Varicella immunity and clinical disease in HIV-infected adults. South Med J. 1994 Jan;87(1):74-6)

How does SARS-CoV-2 compare? Can it be reverse transcribed?

- A study found that **SARS-CoV-2 RNA can be reverse-transcribed and integrated into the DNA of infected human cells** in culture using other enzymes i.e. SARS-CoV-2 can integrate pieces of its genetic code into the human genome. They also found that about 17% of human DNA comprised long interspersed nuclear elements-1 (LINE-1), which are mostly remnants of ancient viruses. LINE-1 elements can produce reverse transcriptase when activated, suggesting a mechanism for incorporation of SARS-CoV-2 into the human genome.
- **The researchers expressed concern that SARS CoV-2 "might haunt our cells long after the infection is gone";** this reverse transcription could be giving SARS-CoV-2 open access to our DNA.
- This could explain why some people test positive for SARS-CoV-2 RNA sometimes months after recovering from their initial infection, even though no infectious virus can usually be grown.
- **Others do not agree with Zhang findings. This is not on scientific grounds** but because although published in a reputable peer-reviewed journal, the study has not yet been replicated, and there is fear that the results, if widely known, would make the public more vaccine-hesitant.

(Zhang L, et al, Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues; PNAS, 2021; 118 (21) e2105968118; https://www.sciencealert.com/we-have-the-strongest-evidence-yet-that-sars-cov-2-can-insert-itself-into-our-genome; https://www.medpagetoday.com/special-reports/exclusives/92632)

Can SARS-CoV-2 disable tetherin?

- The SARS-CoV-2 **spike protein downregulates tetherin** to aid its release from cells and enhance viral spread. Loss of tetherin from cells caused an increase in SARS-CoV-2 viral titre. (Stewart, https://www.biorxiv.org/content/10.1101/2021.01.06.425396v1)

Is SARS-CoV-2 immunosuppressive?

- Although an activated innate immune response was observed in SARS-CoV-2-infected patients, their **immune system failed to launch robust interferon (IFN) responses**, which could potentially lead to increased establishment of viral infection at early stages and contribute to the pathogenicity of COVID-19. An in vitro study indeed revealed that **insufficient IFN responses result in higher viral loads in host cells**. There are limited reports regarding the mechanisms underlying the inhibition of IFN responses by SARS-CoV-2.
- In addition, while neutralising antibodies provide antiviral immunity, **non-neutralising antibodies could enhance SARS-CoV-2 infection through antibody-dependent enhancement (ADE)**, which can induce sustained inflammation, lymphopaenia and cytokine release syndrome, contributing to disease severity.

(Wang C, et al. The Impact of SARS-CoV-2 on the Human Immune System and Microbiome. Infectious Microbes & Diseases. 2020;3(1):14-21)

Neutralising HIV antibodies offer cross-protection to SARS-CoV-2 spike protein

- **HIV neutralising antibodies can effectively bind to the glycosylated spike protein of SARS-CoV-2 to help neutralise it.** (Acharya P, et al. A glycan cluster on the SARS-CoV-2 spike ectodomain is recognized by Fab-dimerized glycan-reactive antibodies. bioRxiv [Preprint]. 2020 Jun 30:2020.06.30.178897)

To summarise:

- SARS-CoV-2 is found in viral reservoirs, particularly the gut.
- SARS-CoV-2 can be reverse transcribed (and hence may already be in the human genome). But this study is controversial.
- SARS-CoV-2 is immunosuppressive, reducing the interferon response and thereby increasing viral load and the level of non-neutralising antibodies.
- SARS-CoV-2 can disable the interferon-induced protein, tetherin, whose expression blocks the release of enveloped viral particles. Disabling tetherin enhances viral spread.
- Hence SARS-CoV-2 can also be said to have retrovirus associations, as with hepatitis B, Herpes simplex viruses (HSV), other Herpes viruses, Ebola, Zika, Epstein Barr and varicella zoster (chickenpox, shingles).
- This tends to support Luc Montagnier's paper showing that RNA fragments of retroviruses were found in the SARS-CoV-2 genome. (https://www.researchgate.net/publication/342926066_COVID-19_SARS_and_Bats_Coronaviruses_Genomes_Peculiar_Homologous_RNA_Sequences_Jean_Claude_perez_Luc_Montagnier)

The immune system: predictors of Long COVID

- A study profiled the longitudinal immune response in individuals who had mild COVID **3-4 months after symptom onset**:
- **In patients who did not go on to suffer Long COVID**, acute infection was characterised by **vigorous coordinated innate and adaptive immune activation, with inflammatory cytokine signalling, stronger interferon (IFN) responses** and a potential IFN plasmablast regulatory circuit; in these patients, **the immune and inflammatory response quickly subsided.**
- **In Long COVID patients**, **the acute response was characterised by dampened IFN and antiviral responses coupled with prolonged inflammatory cytokine signalling** in innate immune cells. Other biomarkers suggest ongoing cellular stress and immune cell activation and differentiation.

(Talla, https://www.biorxiv.org/content/10.1101/2021.05.26.442666v3)

A closer look at interferon (IFN) and viruses

- Antiviral cytokines can be divided into two types: type 1 (comprising interferons and tumour necrosis factor) and type 2 (comprising interleukins). These cytokines can orchestrate antiviral responses through altering the expression of major histocompatibility complex (MHC) molecules, adhesion molecules and co-stimulatory molecules and activating natural killer (NK) cells, cytotoxic lymphocyte (CTLs) and antibody-mediated virus clearance.
- Interferons (IFNs) are a group of glycoproteins which play important roles in preventing viral infections and in activating the host immune system to fight disease in response to pathogens. Based on their location on human chromosomes, together with their amino acid sequences, physicochemical properties and biological characteristics, interferons can be classified as types I, II and III interferons. Type I interferon can make cells resistant in viral infections; Type II interferon plays an essential role in the function of overall immune system; Type III interferon is a novel member of interferon family, which is mainly secreted from virus infected cells.
- Interferons divide into alpha (α), beta (β) and gamma (γ). Interferon α and β play an important role in fighting viruses, while interferon γ's role is regulating inflammation.
- Interferons (IFNs) act as central liaison between the innate and adaptive immune systems, regulating the activation and functions of various immune cell populations. interferons do not directly inhibit the ability of a virus to multiply but, rather, stimulates the infected cells and those nearby to produce proteins that prevent the virus from replicating within them. This inhibits any further production of the virus and thus stops the infection. Interferon also interferes with the growth and multiplication of foreign invaders, for which it was named. The release of interferon can stimulate hundreds of genes, which primarily serve to limit further virus spread and infection. Interferon can also directly stimulate phagocytosis and dendritic cell maturation.
- Several beta-coronavirus (including SARS-CoV-2) proteins are known to inhibit IFN; IFN production was also delayed in SARS-CoV-1, that led to the accumulation of highly activated macrophages in the lungs.

(Acharya D et al. Dysregulation of type I interferon responses in COVID-19. Nature reviews. Immunology, 20(7), 397–398; He Z et al. (2016). Natural products: The master regulators of antiviral cytokines. Current Organic Chemistry. 21. 1-1. 10.2174/1385272821666161214123909; https://naturalinterferon.blogspot.com/)

So what is the relevance of interferon (IFN) in COVID-19?

- **During severe SARS-CoV-2 infection there is robust production of pro-inflammatory cytokines and chemokines, with limited production of IFNs. Minimal amounts of IFNs have been detected in the peripheral blood or lungs of patients with severe COVID-19.**
- A large international study showed that >10% of patients with life-threatening COVID-19 pneumonia had **neutralising antibodies against IFN** at the onset of critical disease. **These inhibit the ability of IFNs to block SARS-CoV-2 infection**. High levels of antibodies to type I interferons (IFNs) have been found with severe COVID-19. (Autoimmune Registry)
- **SARS-CoV-2 encodes several proteins that specifically evade initial type I interferon (IFN) response**. In severe and critical COVID-19 patients a highly impaired IFN type I response was observed, associated with a persistent blood viral load and an exacerbated inflammatory response.
- SARS-CoV-2 can disable tetherin, which is an interferon-induced protein and is known as an antiviral restriction factor.
- In severe COVID-19 patients, their immune system failed to launch robust interferon (IFN) responses.
- An *in vitro* study revealed that insufficient IFN responses result in higher viral loads in host cells.

(Wang C, et al. The Impact of SARS-CoV-2 on the Human Immune System and Microbiome. Infectious Microbes & Diseases. 2020;3(1):14-21; Hadjadj J, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science 2020;369; Acharya D et al. Dysregulation of type I interferon responses in COVID-19. Nature reviews. Immunology, 20(7), 397–398; Bastard P, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science. 2020 Oct 23;370(6515):eabd4585)

Interferon as a pharmaceutical treatment

- Interferon is effective as a pharmceutical but can have several side effects, including fatigue and headaches. In some instances it could also lead to low thyroid activity, low platelet count and depression.(Lei X, et al. Activation and evasion of type I interferon responses by SARS-CoV-2. Nat Commun 2020;11:3810)
- To combat this, a large-scale trial of a new treatment it is hoped will help stop Covid-19 patients from developing severe illness has begun in the UK.
- It involves inhaling the protein interferon beta, which the body produces when it gets a viral infection.
- The hope is it will stimulate the immune system, priming cells to be ready to fight off viruses.
- Early findings suggested the treatment cut the odds of a Covid-19 patient in hospital developing severe disease - such as requiring ventilation - by almost 80%.

(https://www.bbc.co.uk/news/health-55639096)

Raising interferon production naturally

- Epigallocatechin gallate (EGCG)
- Quercetin
- Allicin (from garlic)
- Glutamine
- Probiotics (Lactobacillus rhamnosus GG)
- Vitamin E
- Glycine
- Astragalus
- Siberian Ginseng
- Co-enzyme Q10
- Echinacea
- Ginkgo
- Liquorice
- Melatonin
- Milk thistle
- Vitamin C and bioflavonoids
- Medicinal Mushrooms: Reishi, Maitake, Shiitake, Kombucha and others

Something missing?

- **IFN induction can be enhanced by vitamin D**. (Feng X, et al. Vitamin D enhances responses to interferon-β in MS. Neurol Neuroimmunol Neuroinflamm. 2019 Oct 3;6(6):e622; Fabri M, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. Sci Transl Med. 2011 Oct 12;3(104):104ra102).
- **Vitamin D, magnesium and zinc are essential in the modulation of the interferon (IFN) signalling pathway.** (Nabi-Afjadi M, et al. The effect of vitamin D, magnesium and zinc supplements on interferon signaling pathways and their relationship to control SARS-CoV-2 infection. Clin Mol Allergy. 2021 Nov 8;19(1):21)
- Vitamin D, zinc and glutamine have been shown to facilitate immune responses mediated by IFN signalling. (Name JJ, et al. Vitamin D, zinc and glutamine: Synergistic action with OncoTherad immunomodulator in interferon signaling and COVID-19 (Review). Int J Mol Med. 2021 Mar;47(3):11)
- Increasing dietary vitamin D3 content partially regulated the expression of VD3/VDR-type I interferon axis genes after immune challenge. (Cheng K, et al. Vitamin D3 modulates yellow catfish (Pelteobagrus fulvidraco) immune function in vivo and in vitro and this involves the vitamin D3/VDR-type I interferon axis. Dev Comp Immunol. 2020 Jun;107:103644)

Conclusions

- There are many potential mechanisms underlying Long COVID. There is no one mechanism involved and there may be several mechanisms in operation at the same time. There may be many more than I have listed.
- A likely, but relatively unexplored, candidate is reactivation of viruses (SARS-CoV-2 and others) from viral reservoirs.
- This explanation allows for the initiation or continuation of viral symptoms, as well as the remitting and relapsing nature of Long COVID. It also allows for patients to be PCR negative (in the case of SARS-CoV-2 reactivation), unless the viral reservoir is in the upper respiratory tract.
- In common with some other 'retrovirus-associated viruses', SARS-CoV-2 can (probably) insert itself in the human genome via reverse transcriptase, is immunosuppressive, and can disable tetherin, all of which enable viral spread.
- Immunosuppression and the disabling of tetherin prevent an appropriate interferon response to the virus. The interferon response may be the determining factor in whether a COVID-19 patient develops Long COVID or not.
- Various natural substances, particularly vitamin D, can boost interferon production.