mRNA VACCINE INDUCED DAMAGE MECHANISMS

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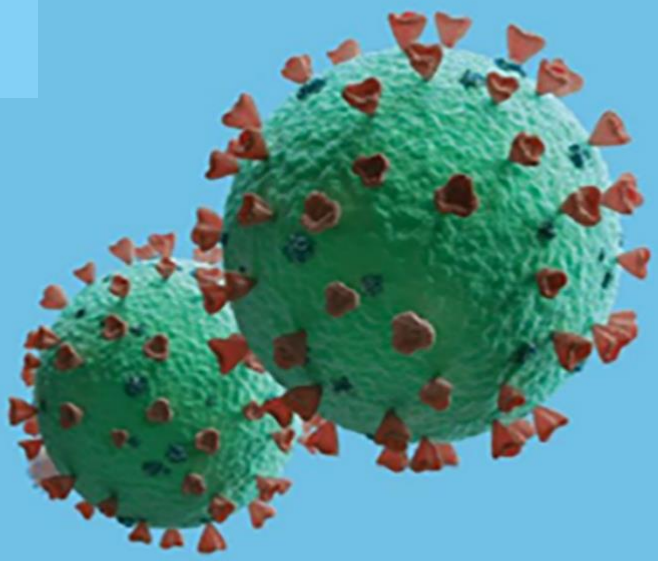

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mRNA VACCINE INDUCED DAMAGE MECHANISMS

The various mechanisms by which COVID-19 vcaccines can induce immunopathologies include the following:

ANTIBODY-DEPENDENT ENHANCEMENT (ADE). [1]

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However, ADE has also been explored in depth in MERS [3], Dengue [4], Zika virus [5], Ebola [6], HIV [7] and seasonal influenza [8].

The ADE mechanism is quite complex but can be summarized as follows: when a subject who possesses a sub-optimal antibody level (as a result of primary infection or vaccination) comes into contact with a similar virus and becomes infected, his **immune system promotes infection and fatal complications of the disease.** In other words, **a proportion of the vaccinated are predisposed by the vaccination to developing serious and fatal complications of the very disease they are meant to be protected from.**

When the virus infects macrophages, instead of being processed for presentation to other cells of the immune system, on the one hand the virus inhibits type I IFN signaling and on the other hand it enables the pro-inflammatory expression of IL-1, IL-6, and TNF-α, contributing to **cytokine storm syndrome** and potentiation for **fatal disease**. In these cases, the development of acute respiratory disease coincides with antiviral IgG seroconversion.

It is important to emphasize that disease potentiation manifests as severe and potentially fatal lung disease both following natural infection in predisposed individuals and especially following vaccination. However, the two cases are distinguishable, as post-infectious ADE pneumonia presents predominantly in macrophage infiltration, whereas post-vaccinal ADE pneumonia is eosinophilic in nature.[9]

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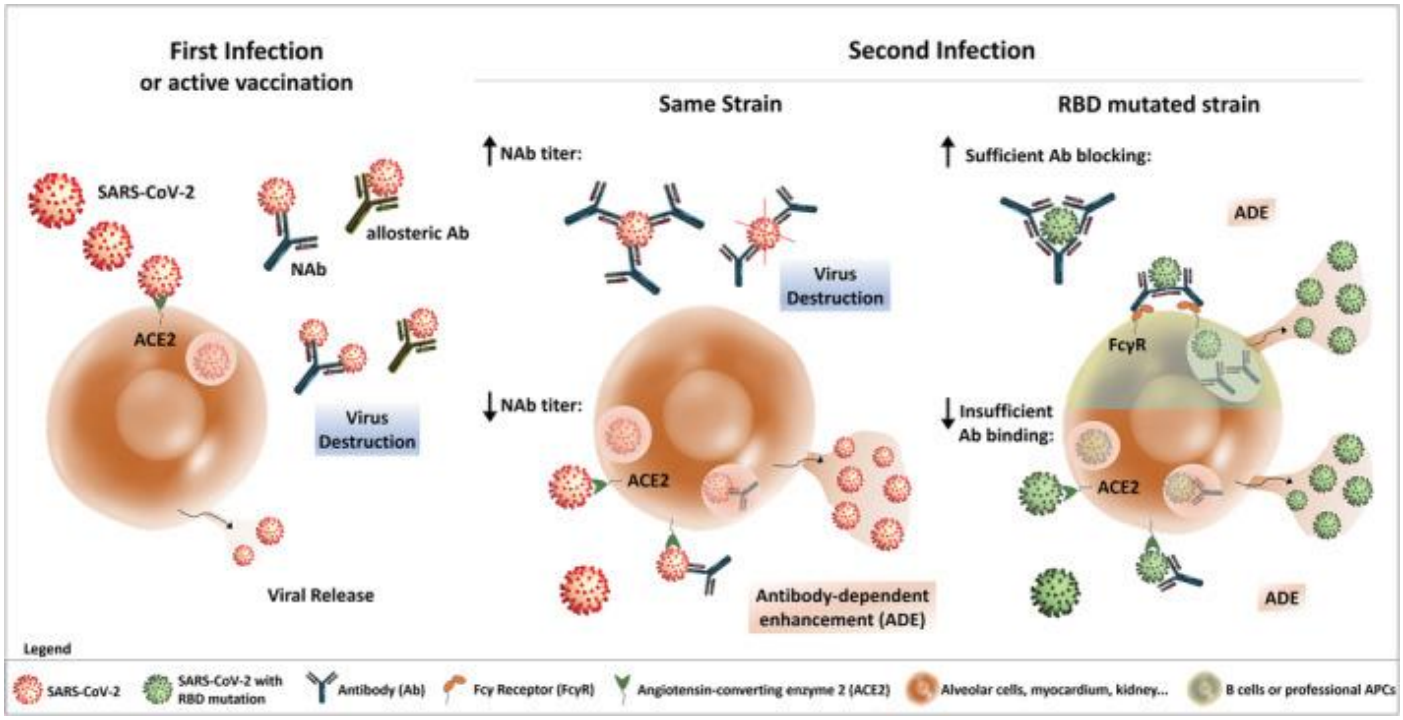
COVID-19-related inflammatory responses could **also** be induced by **dysregulation of the complement system**, a critical component of host innate immunity.
A subset of patients with COVID-19 has been known to develop vasculitic lesions**, blood vessel occlusion, and infarcts**. Histopathologic reports from tissue sections suggest features associated with immune complex-mediated vasculitis, including infiltration of monocytes and lymphocytes in and around blood vessels, wall thickening, and focal hemorrhage.[10]
From the perspective of the mechanism inducing harm, the severe/fatal complications associated with SARS-Cov-2 infection can be considered a consequence of ADE.
ADE explains why the **elderly people are at greater risk** than children and healthy adults, because **they have more non-neutralizing antibodies from coronavirus infections or vaccinations (e.g., flu shots), and have an immune system that is inefficient at fighting infection. Pregnant women and infants under one year of age are also susceptible to disease enhancement if they are reinfected.**
Becuase of its ability to form **quasispecies** (see below), Sars-Cov-2, could certinaly be responsible for the phenomenon of disease enhancement in vaccinees, which should have been investigated and excluded before proceeding with human trials.



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Potential pathways of aggravation of SARS-CoV-2 infection by ADE. Initially, the spike protein of SARS-CoV-2 binds through the RBD to angiotensin-converting enzyme 2 (ACE2) on the host cell surface for virus invasion. After some days, humoral responses develop against the virus, eliminating infection through allosteric and neutralizing antibodies (NAbs). After a second infection with the same SARS-CoV-2 strain virus destruction may occur, if the NAb titer is high enough. However, in case of a low NAb titer, ADE may be observed following binding of the SARS-CoV-2/antibody complex to ACE2, internalization of the complex and IgG induced stimulation. In addition, immunized patients may be re-infected with a different SARS-CoV-2 strain, such as a RBD-mutated strain. In this context, already existing Abs could bind with reduced affinity to mutated RBD, inducing low levels of SARS-CoV-2/antibody complexes, following by internalization through the ACE2 receptor and ADE. On the other hand, immunized patients re-infected by an RBD-mutated strain may present sufficient Ab blockade of the heterotypic SARS-CoV-2 strain. In this case, SARS-CoV-2 covered by Abs may connect to Fcγ receptors II (FcγRII) on the surface of B cells or other professional antigen-presenting cells (APCs). This receptor mediates SARS-CoV-2 invasion into immune cells, further spreading viral infection into all organs and ADE.

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To date, only a few partial in vitro and in vivo studies are available [11] suggesting risk in humans. Although the issue was brought to the attention of the EMA back in March 2020 by the Coalition for Epidemic Preparedness Innovations (CEPI) and the Brighton Collaboration (BC) Safety Platform for Emergency vACcines (SPEAC) [12] there are still no useful available results.

AUTOIMMUNE INFLAMMATORY SYNDROME

The presence of autoantibodies in patients who have developed Covid-19 may suggest that an autoimmune/inflammatory mechanism may be an additional event determining the severity of the disease. [13]

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It would appear that the source of this **adverse vaccine reaction, which has already been encountered among people vaccinated with "Pfizer" vaccine** [14], and of serious complications reported in the literature following COVID-19 [15], is a molecular mimicry mechanism**, i.e. of sequence homology (similarity) between Spike proteins and human proteins from various tissues.**

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In this regard, a recent study by **Prof. Kanduc and Prof. Shoenfeld** attempted to answer by way of a **bioinformatic proteomics stud** the question why SARS-CoV-2 attacks the respiratory system so aggressively. Their results report extensive peptide sharing between the SARS-CoV-2 spike glycoprotein and lung surfactant-related proteins (13 of 24 peptides). **On the basis of these findings, the authors highlight the risk of using vaccines containing the full-length spike of SARS-Cov-2**.[16]

Equally, **Dr. JL Wyler**, studied the sequence homology between SARS-Cov-2 proteins and human proteins and confirmed **the high sequence homology and potential risk of autoimmunity.**

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He also hypothesizes that exposure to these specific peptides, either through infection or vaccination, could cause enhanced pathogenicity as a result of future exposure due to new pandemics, or outbreaks of infection, or through mass vaccination programs on a global scale, and because this phenomenon is plausible, he felt that it essential to evaluate it before proceeding with the use of vaccines on humans against SARS-Cov2. [17]
Finally, another research group [18] tested 5 different blood samples positive for IgG and IgM antibodies to SARS-CoV-2 [19]. This study showed **that 21 of 50 tissue antigens had moderate to strong reactions with antibodies to SARS-CoV-2. This finding is a sufficiently robust indication of a cross-reaction between SARS-CoV-2 proteins and a variety of tissue antigens, in addition to lung tissue, that could lead to autoimmunity against connective tissue, the cardiovascular, gastrointestinal, and nervous systems, even in the medium to long term, after resolution of acute disease.[20]**

VACCINE RESISTANCE

This phenomenon is related to the formation of mutant **populations called quasispecies, typical of single-chain RNA** viruses, also evident in SARS-Cov-2. [21]

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Viral quasispecies are defined as 'collections' of closely related viral genomes that undergo a continuous process of genetic variation, competition among generated variants and selection of the most suitable distributions in a given environment. [22] These mutant distributions (also referred to as 'mutant swarms' or 'mutant clouds') are generated in infected cells and organisms as a result of the replication of RNA viruses and some DNA viruses.

It is important to note that by using speically developed mathematical models, it has been possible to verify that viral quasi-species are not simply a collection of different mutants, but a **group of interactive variants**, which together contribute to the characteristics of the population. [23]

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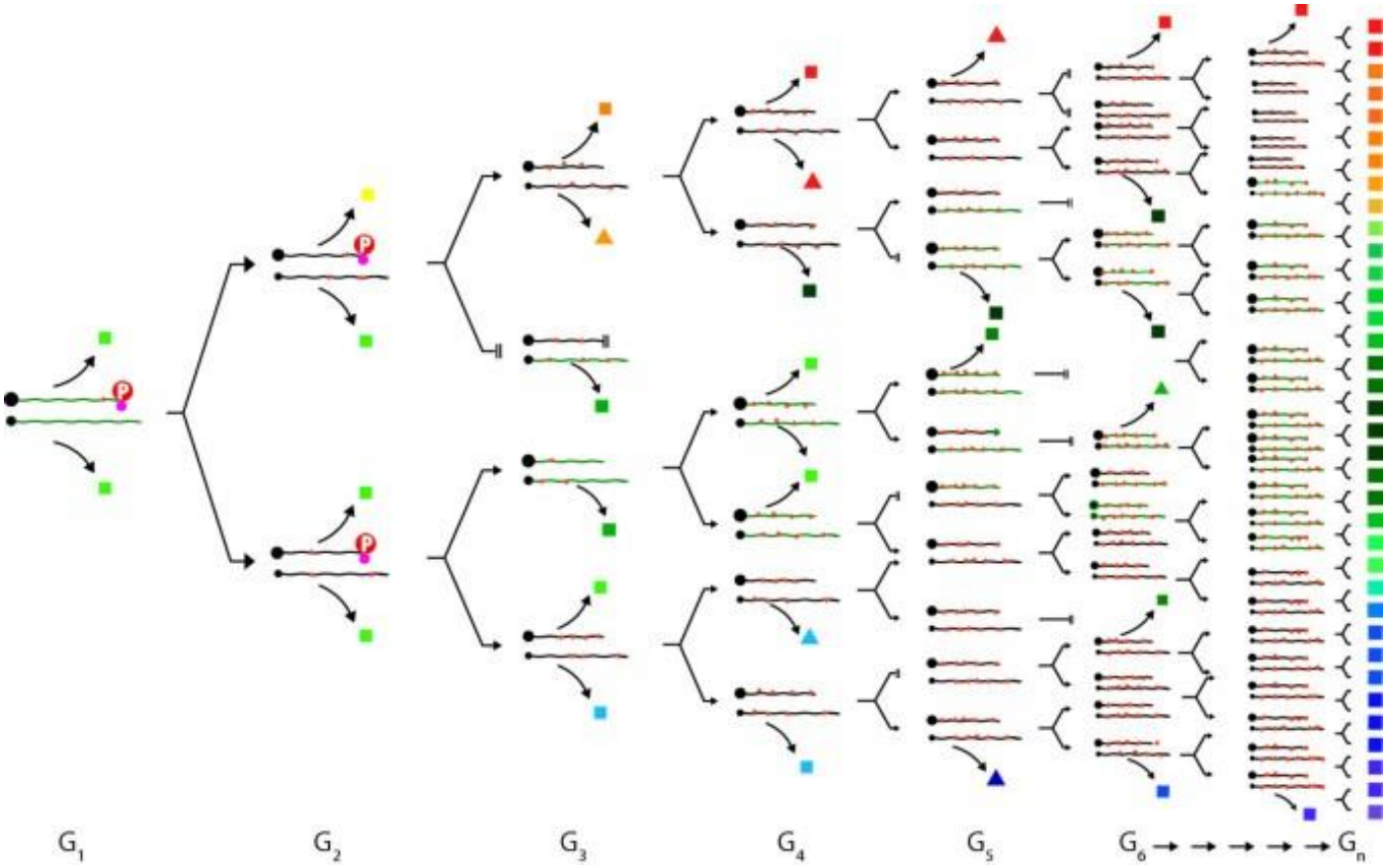

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For the explanation see the text of the article

Mutant spectra are the source of virus adaptability because they constitute dynamic (continuously evolving) repositories of genotypic and phenotypic viral variants.
Such molecular dynamism derives from the limited copying fidelity exhibited by RNA-dependent RNA polymerases (also referred to as RNA replicases), RNA-dependent DNA polymerases (or reverse transcriptases, RTs), and some DNA-dependent DNA-polymerases.

Error rates for RNA viruses, retroviruses, and some DNA viruses are in the range of 10^{-3} to 10^{-5} mutations introduced per copied nucleotide, whcih is from 10^{4} to 10^{6} times higher than those that occur during normal cellular chromosomal DNA replication.

High error rates underlie quasispecies dynamics, which is characterized by two main properties:
(1) **Viruses replicate as a set of variant genomes** (called mutant spectra, distributions, clouds, or swarms) that differ from each other in one or more mutations.
The average number of mutations per genome in a mutant spectrum is a measure of the genetic complexity of the viral population, which is a multifactorial parameter.
(2) The main consequence of viruses replicating as mutant spectra is that **there is no "sequence" or "genome" of an isolated virus**, as it is still misleadingly described with the nucleotide sequence of the viral genome reported in databases and still in some publications**. The reality is that viral populations are mixtures of mutants, contrary to what might be assumed when only consensus sequences are considered.**

A virus that spreads in nature can be the result of direct contact between infected and susceptible hosts (host-to-host) or a more complex series of events such as virus transfer from infected hosts to external objects (object-to host) that in turn become a source of virus.
At each stage of host-to-host or object-to host transmission uncertainties derived from the presence of multiple genome types serve to limit the predictability of evolutionary outcomes.

Furthermore, for any virus, the invasion of a new susceptible host is a new beginning in terms of evolutionary events.

Whenever the host receives a nonidentical set of infectious genomes, they face a unique set of selective pressures, studied through phylodynamics, i.e., through analysis of the immunological, epidemiological, and evolutionary forces that influence the phylogenetic trees of virus genomes isolated from infected individuals [24].
The presence of an abundant number of mutant genomes within a population gives viruses the potential for **rapid evolution, which can lead to various consequences, including disease enhancement due to weak antibody formation, as seen above, and vaccine resistance** [25].
The presence of quasi-species also in vaccines from live attenuated viruses has already been described in the literature.
The genomic sequence and the biological characteristics of quasi-species subpopulations may differ significantly, and the selection of a more virulent subpopulation in the vaccinated host may have adverse consequences.

For example, the virus contained in the mumps vaccine has been documented to be a distribution of near-species mutants, and neurovirulence has been associated with changes in the level of genetic heterogeneity at specific genomic sites [26].
The Urabe strain formerly used in the mumps vaccine (no longer used because of high neuro-virulence), contains subpopulations that differ in the sequence of the gene for hemagglutinin-neuroaminidase and in the propensity to cause post-vaccinal meningitis [27]. Regarding the varicella vOka DNA vaccine virus, it has been known since 2002 that it is not a single virus but a viral mixture [28] .

NON-IGE MEDIATED PSEUDOALLERGY (CARPA)
The delivery system is a key element in mRNA vaccines, as it should protect the mRNA from RNases and enhance delivery into the cytosol. In the case of COVID-19 vaccines, lipid nanoparticles (LNPs) are mainly used as mRNA vehicles. LNP complexes are the most widely used platform and present the best results in mRNA delivery.
LNPs are mainly composed of ionizable lipids, cholesterol, phospholipids, and polyethylene glycol (PEG) anchored to lipids and in addition to their role in mRNA protection, they facilitate cellular uptake, enhance exit from endosomes, and allow release into the cytoplasm. LNPs may also protect mRNA molecules from being recognized in endosomes by TLRs, preventing excessive activation of the innate immune system.

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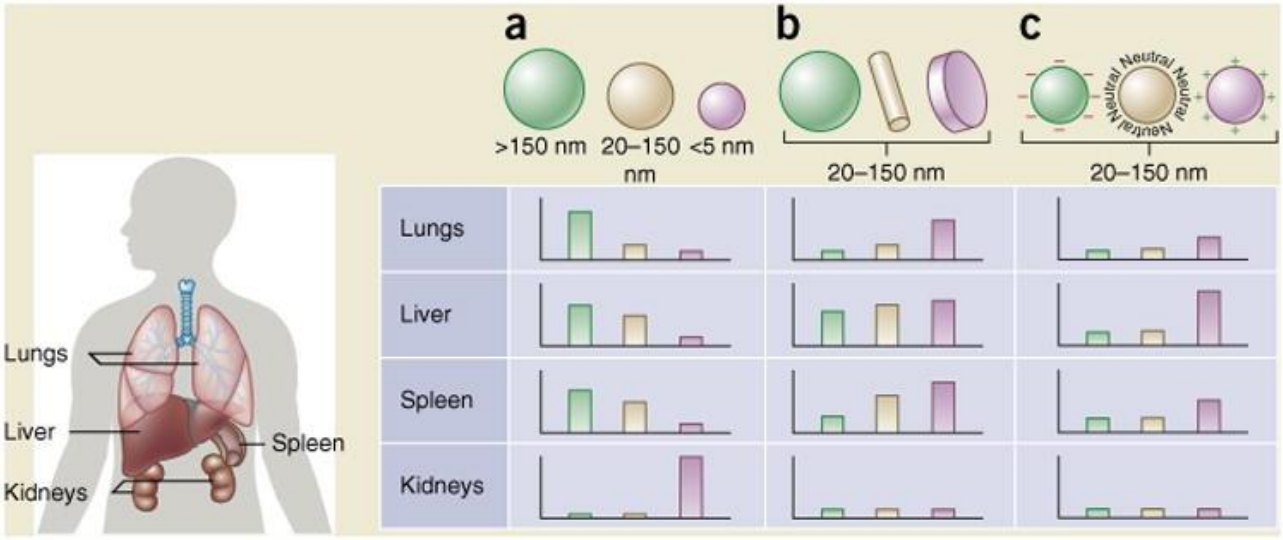
However, there are some critical issues remain that need to be overcome in the administration of mRNA vaccines, [29] such as:

- the toxicity of some lipid formulations;
- the difficulty in reaching immune cells in dedicated secondary lymphoid organs; and
- the need to tailor each delivery system to the route of administration.

From a toxicological point of view, it should be mentioned that studies of RNAi release through cationic LNPs (nanoparticle liposomes) showed that polyamines such as polyethylenimine and poly-L-lysine led to high serum liver enzyme levels, reduced body weight, and dramatically reduced total leukocyte counts, suggesting a **mechanism of immunosuppression after intravenous administration**.[30]



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Nanoparticle size, shape and surface charge dictate biodistribution among the different organs including the lungs, liver, spleen and kidneys. (**a**) Spherical particles, including gold nanoparticles, liposomes and polymeric micelles/nanoparticles can vary in size and display disparate *in vivo* fates. Large rigid particles with diameters >2,000 nm accumulate readily within the spleen and liver, as well as in the capillaries of the lungs. Nanoparticles in the range of 100–200 nm have been shown to extravasate through vascular fenestrations of tumors (the EPR effect) and escape filtration by liver and spleen. As size increases beyond 150 nm, more and more nanoparticles are entrapped within the liver and spleen. Small-sized nanoparticles (<5 nm) are filtered out by the kidneys. [31]

(**b**) Novel 'top-down' and 'bottom up' fabrication techniques have enabled the exploration of different geometries of nanoparticles, including cylindrical and discoidal shapes, which have been shown to exhibit pronounced effects on pharmacokinetics and biodistribution. Different nanoparticle shapes exhibit unique flow characteristics that substantially alter circulating lifetimes, cell membrane interactions and macrophage uptake, which in turn affect biodistribution among the different organs. [32]

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(**c**) Charge of nanoparticles stemming from distinct surface chemistries influences opsonization, circulation times and interaction with resident macrophages of organs comprising the MPS, with positively charged particles more prone to sequestration by macrophages in the lungs, liver and spleen. Neutral and slightly negatively charged nanoparticles have longer circulation lifetimes and less accumulation in the aforementioned organs of the MPS.[33]
In both **b** and **c**, the size of the nanoparticles is assumed to range from 20–150 nm. Individual panels represent *in vivo* fates of nanoparticles, taking into account singular design parameters of size, shape and surface charge independent of one another, and for this reason, respective scales vary from one panel to the next. It is important to note that *in vivo* biodistribution will undoubtedly vary based on the interplay of several of the above parameters.

It is also known that intravenous injection of a variety of nanotechnology-enhanced (liposomal, micellar, polymer conjugated) and protein-based (antibodies, enzymes) drugs can lead to hypersensitivity reactions (HSR), also known as **infusion or anaphylactoid reactions**. The molecular mechanism of mild to severe allergy symptoms may differ from case to case and is mostly unknown, although in many cases a major cause or contributing factor is an **activation of the complement system** (C).

The clinical relevance of C activation-related HSR, a non-IgE-mediated pseudoallergy (CARPA), lies in its **unpredictability** and **occasional lethal outcome**. Consequently, there is a medical need to develop laboratory analyses and animal models to quantify CARPA.
It is emphasized that anaphylatoxin-induced mast cell release may not fully explain severe reactions; it may also contribute a "second hit" on allergy-mediating cells and it is suggested that CARPA represents a **"blood stress" reaction**, a systemic struggle of the body against harmful biological and chemical agents through the anaphylatoxin/mast cell/circulatory system axis, in analogy to the body's struggle against physical and emotional stress through the hypothalamus/pituitary/adrenal axis.
In both cases, the response to a wide variety of harmful effects is channeled into a uniform pattern of physiological changes.[34]

[33] Xiao K, Li Y, Luo J, et al.
The effect of surface charge on in vivo biodistribution of PEG-oligocholic acid based micellar nanoparticles.
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Blood cell changes in complement activation-related pseudoallergy
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https://www.degruyter.com/document/doi/10.1515/ejnm-2015-0021/html

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Features of complement activation-related pseudoallergy to liposomes with different surface charge and PEGylation: comparison of the porcine and rat responses.
J Control Release. 2014 Dec 10;195:2-10. doi: 10.1016/j.jconrel.2014.08.009.
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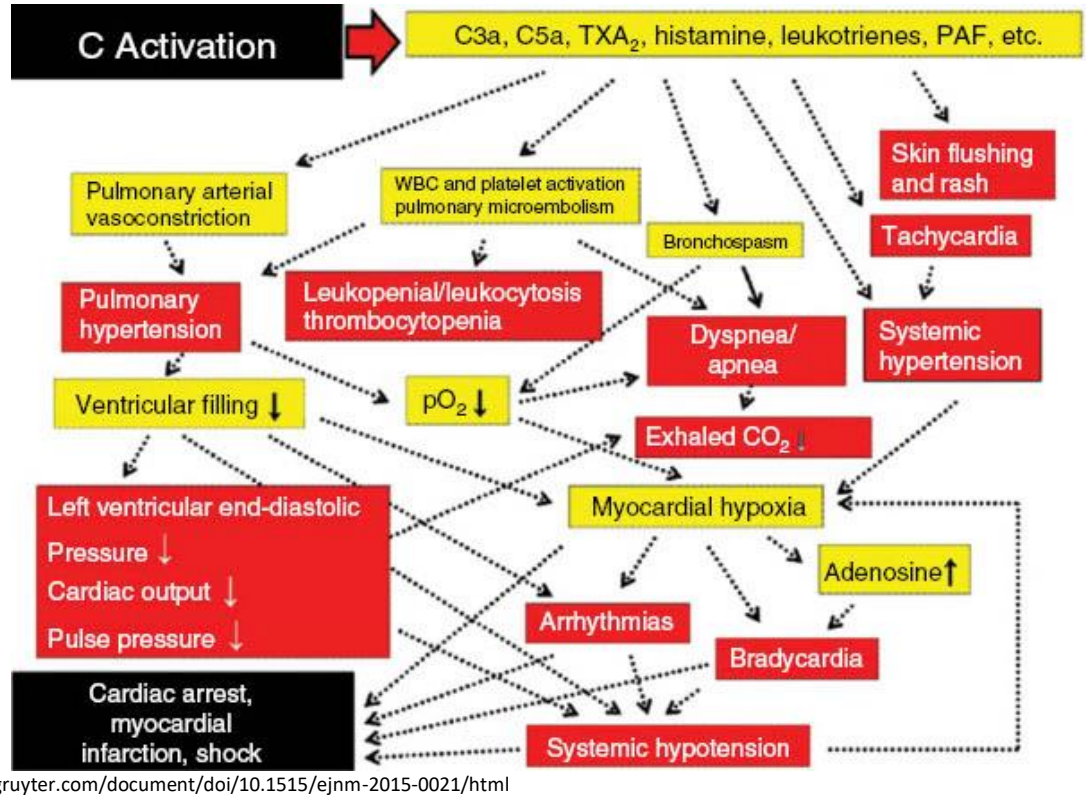

https://www.degruyter.com/document/doi/10.1515/ejnm-2015-0021/html
Interrelationships between different abnormalities during CARPA, leading to clinical symptoms.

The liposome that forms the vehicle for the Pfizer vaccine contains two new excipients: cationic lipid ALC-0315 containing a tertiary amine and two esterified portions, and PEGylated lipid ALC-0159 containing an acetamide functional group. Both compounds are still being studied for toxicology, pharmacokinetics, and pharmacodynamics because they are newly introduced.

It should be noted, however, that in the Pfizer vaccine [35] assessment report, it is indicated that only the entire formulation (modified RNA in the LNPs) was used, so there is no toxicological data on the LNP alone or its specific new excipients. No genotoxicity or carcinogenicity studies were provided because the components of the vaccine formulation are lipids and RNAs that are not expected to have genotoxic potential.

However, the new excipient ALC-0159 contains a portion of acetamide that is potentially genotoxic. The risk assessment performed by the manufacturer indicates that the genotoxicity risk related to this excipient is very low based on literature data reporting the genotoxicity of acetamide following chronic high-dose oral (non-injection) use. However, this assessment is questionable because the injective and oral routes of administration are not comparable in toxicological terms.

The cationic lipid ALC-0315 due to the presence of cationic amine functional groups has the potential to trigger CARPA, however, the manufacturer reports that although systemic complement activation (which can sometimes be induced by liposomal and biological drugs and can cause hypersensitivity reactions) is known, it has not been studied because no signs indicative of such clinical manifestations have been detected.

However, it should be noted that many adverse reactions reported in the pharmacovigilance report [36] indicate this type of induced damage mechanism.

SYSTEMIC REACTIONS

[35] https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf

[36] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/962405/COVID-19_mRNA_Pfizer-_BioNTech_Vaccine_Analysis_Print.pdf

Recent human studies have demonstrated **moderate and in some cases severe systemic or injection site reactions to several mRNA vaccine platforms**. [37]

Potential safety issues that should be evaluated in future preclinical and clinical studies include local and systemic **inflammation, biodistribution and persistence of the expressed immunogen, stimulation of autoreactive antibodies and potential toxic effects of any non-native nucleotides and components of the delivery system**.

A possible concern could be that some mRNA-based vaccine platforms induce potent type I interferon responses associated not only with inflammation but also with **autoimmunity**.[38]

By stimulating dendritic cell maturation and eliciting robust T- and B-cell responses, mRNA vaccines **may activate autoreactive lymphocytes and reactivate autoimmune diseases**. Therefore, **individuals at increased risk for autoimmune reactions should be identified before mRNA vaccination and appropriate precautions should be taken**.[39]

[37] Bahl K, Senn JJ, Yuzhakov O, et al.
Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses.
Mol Ther. 2017;25(6):1316-1327. doi:10.1016/j.ymthe.2017.03.035
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Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: an open-label, non-randomised, prospective, first-in-human phase 1 clinical trial.
Lancet. 2017 Sep 23;390(10101):1511-1520. doi: 10.1016/S0140-6736(17)31665-3. Epub 2017 Jul 25.
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Adjuvant effects of a sequence-engineered mRNA vaccine: translational profiling demonstrates similar human and murine innate response.
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Induction of an IFN-Mediated Antiviral Response by a Self-Amplifying RNA Vaccine: Implications for Vaccine Design.
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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5421303/

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The importance of the type I interferon system in autoimmunity.
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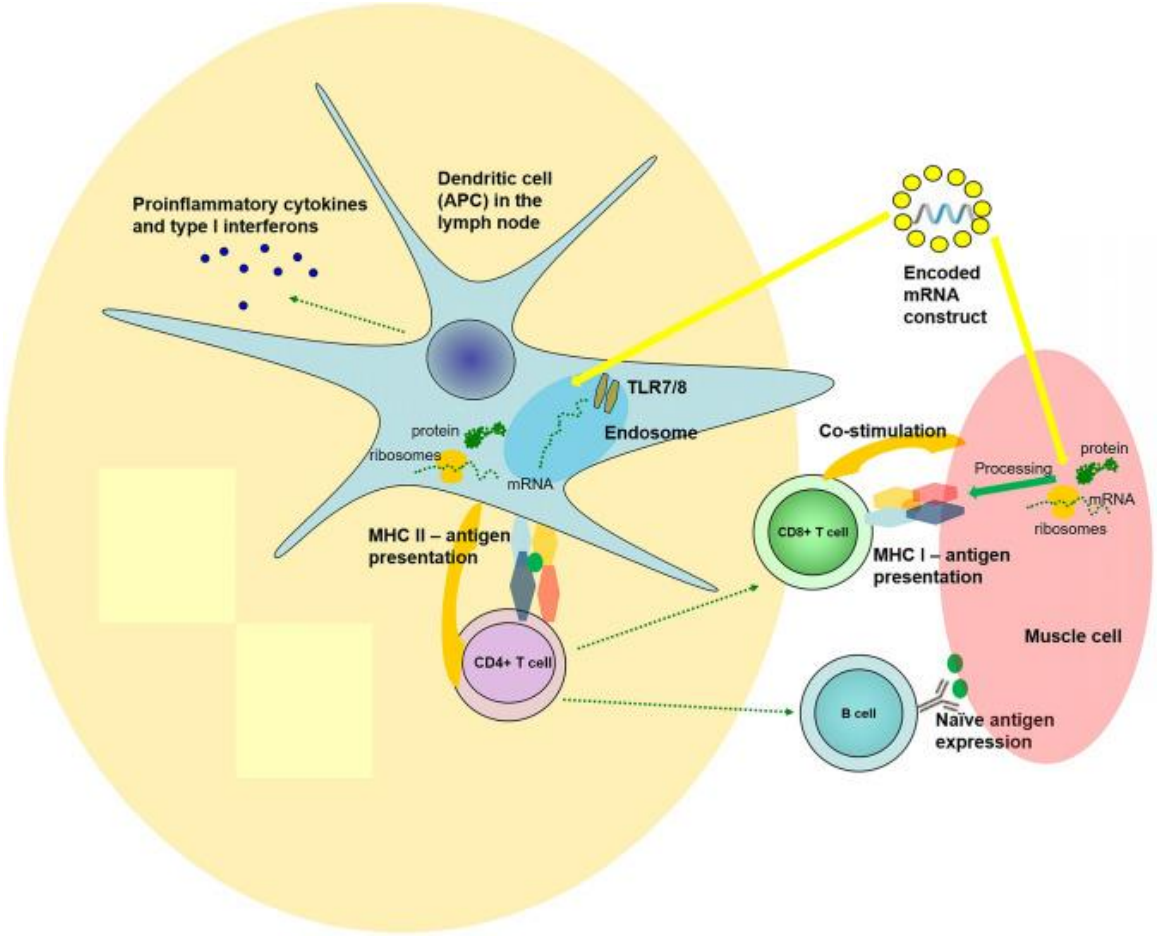
Do COVID-19 RNA-based vaccines put at risk of immune-mediated diseases? In reply to "potential antigenic cross-reactivity between SARS-CoV-2 and human tissue with a possible link to an increase in autoimmune diseases".
Clin Immunol. 2021;224:108665. doi:10.1016/j.clim.2021.108665
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7833091/

Vojdani A, Kharrazian D.
Potential antigenic cross-reactivity between SARS-CoV-2 and human tissue with a possible link to an increase in autoimmune diseases.
Clin Immunol. 2020;217:108480. doi:10.1016/j.clim.2020.108480
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7246018/

Velikova T, Georgiev T.
SARS-CoV-2 vaccines and autoimmune diseases amidst the COVID-19 crisis.
Rheumatol Int. 2021;41(3):509-518. doi:10.1007/s00296-021-04792-9
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7846902/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7846902/
The immune processes involved in the mechanism of mRNA vaccines—activation of T helper cells (CD4+) via MHC I molecules and processed viral antigen in antigen-presenting cells in the lymph node; stimulation of T cytotoxic cells (CD8+) via MHC class II molecules and processed viral antigen and B cell by native viral antigens. In antigen-presenting cells, mRNA sense TLR7 and 8, leading to activation of down cascade and production and secretion of proinflammatory cytokines and type I interferons. Some of the mechanisms are simplified in the figure by omitting (i.e., the inflammasome, the proteasome, secondary messengers, etc.)

Another potential safety issue could arise from the **presence of extracellular RNA during vaccination**. Naked extracellular RNA has been shown to increase the permeability of tightly packed endothelial cells and thus may contribute to edema.[40]
Other studies have shown that extracellular RNA, acting as a DAMPS, also promotes pathological **thrombus** formation, and **cardiomyocyte death** [41].

[40] Fischer S, Gerriets T, Wessels C, Walberer M, Kostin S, Stolz E, Zheleva K, Hocke A, Hippenstiel S, Preissner KT.
Extracellular RNA mediates endothelial-cell permeability via vascular endothelial growth factor.
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Extracellular RNA constitutes a natural procoagulant cofactor in blood coagulation. Proc Natl Acad Sci U S A. 2007;104(15):6388-6393.
doi:10.1073/pnas.0608647104
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1851071/

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Extracellular RNA as a Versatile DAMP and Alarm Signal That Influences Leukocyte Recruitment in Inflammation and Infection.
Front Cell Dev Biol. 2020;8:619221. Published 2020 Dec 18. doi:10.3389/fcell.2020.619221
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New directions in inflammation and immunity: the multi-functional role of the extracellular RNA/RNase system.
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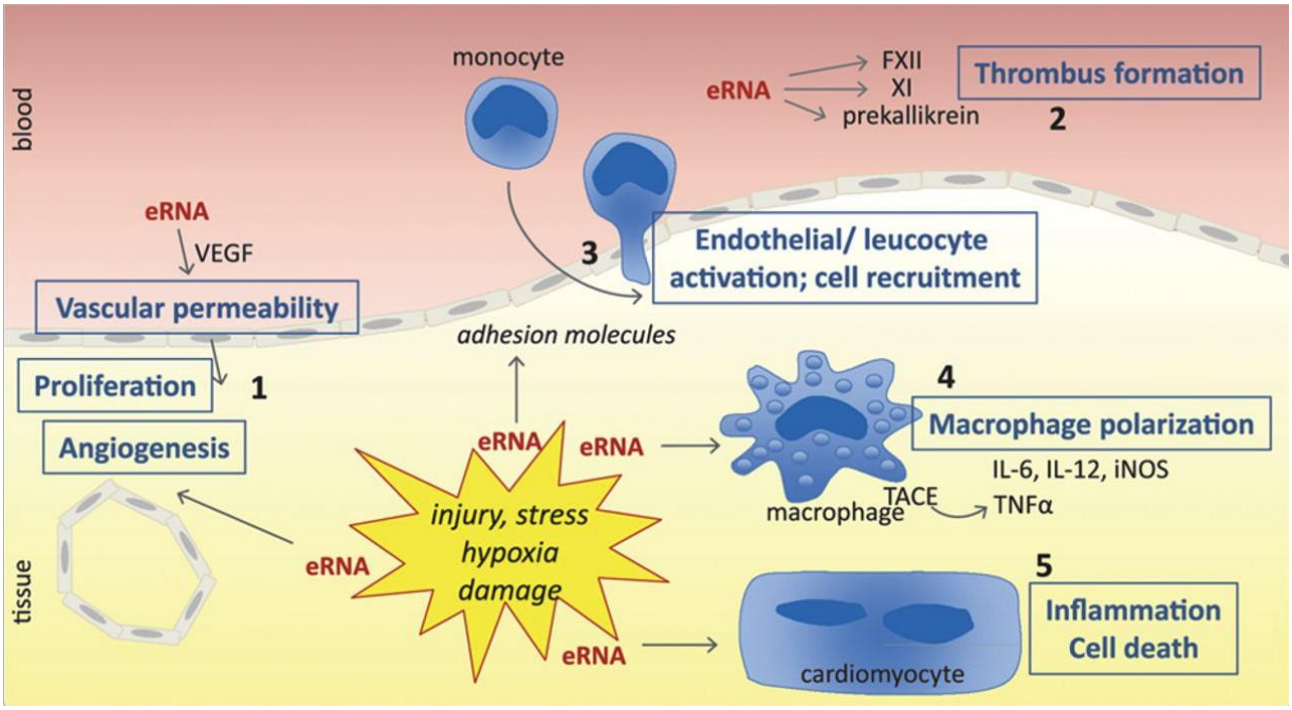
https://www.researchgate.net/publication/42973591_New_Directions_in_Inflammation_and_Immunity_The_Multi-functional_Role_of_the_Extracellular_RNARNase_System



https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.115.307961
Contribution of extracellular RNAs (eRNAs) to vascular homeostasis and cardiovascular pathologies. After cell stress (such as hypoxia, injury, and pathogen exposure), eRNA is found to be expressed at various extracellular sites, both intravascularly and extravascularly. In addition, eRNA may be derived from damaged or apoptotic cells or becomes released on inflammatory cell activation and accumulates within atherosclerotic plaques in the course of disease development. The following functional activities of eRNA are promoted by direct interactions with specific extracellular proteins: (1) promotion of vascular hyperpermeability by eRNA as coreceptor/cofactor of vascular endothelial growth factor (VEGF) and by induction of vasculogenesis/angiogenesis. (2) Initiation of intrinsic blood coagulation (via the contact phase proteins) and thrombus formation. (3) Induction of recruitment of leukocytes to the inflamed vessel wall by eRNA-mediated elevation of expression of intercellular adhesion molecule-1 in endothelial cells or vascular cell adhesion molecule-1 and P-selectin in vascular smooth muscle cells. (4) Skewing of monocytes/macrophages toward the M1 proinflammatory phenotype, accompanied by an upregulated expression of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-12, inducible nitric oxide synthase (iNOS), and response factors, such as chemokines. Here, eRNA triggers TNF-α–converting enzyme (TACE) to liberate the functionally active TNF-α. (5) Promotion of cardiomyocyte death by inducing a hyperinflammatory cascade involving TNF-α in the context of ischemia–reperfusion injury.

Therefore, ongoing safety evaluation is required, as different mRNA systems and modes of administration are used for the first time in humans and are tested on larger and more heterogeneous patient populations.

NEUROLOGICAL DAMAGE ASSOCIATED WITH PRION PROTEIN FORMATION [42]

Zernecke A, Preissner KT.
Extracellular Ribonucleic Acids (RNA) Enter the Stage in Cardiovascular Disease.
Circ Res. 2016 Feb 5;118(3):469-79. doi: 10.1161/CIRCRESAHA.115.307961.
https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.115.307961

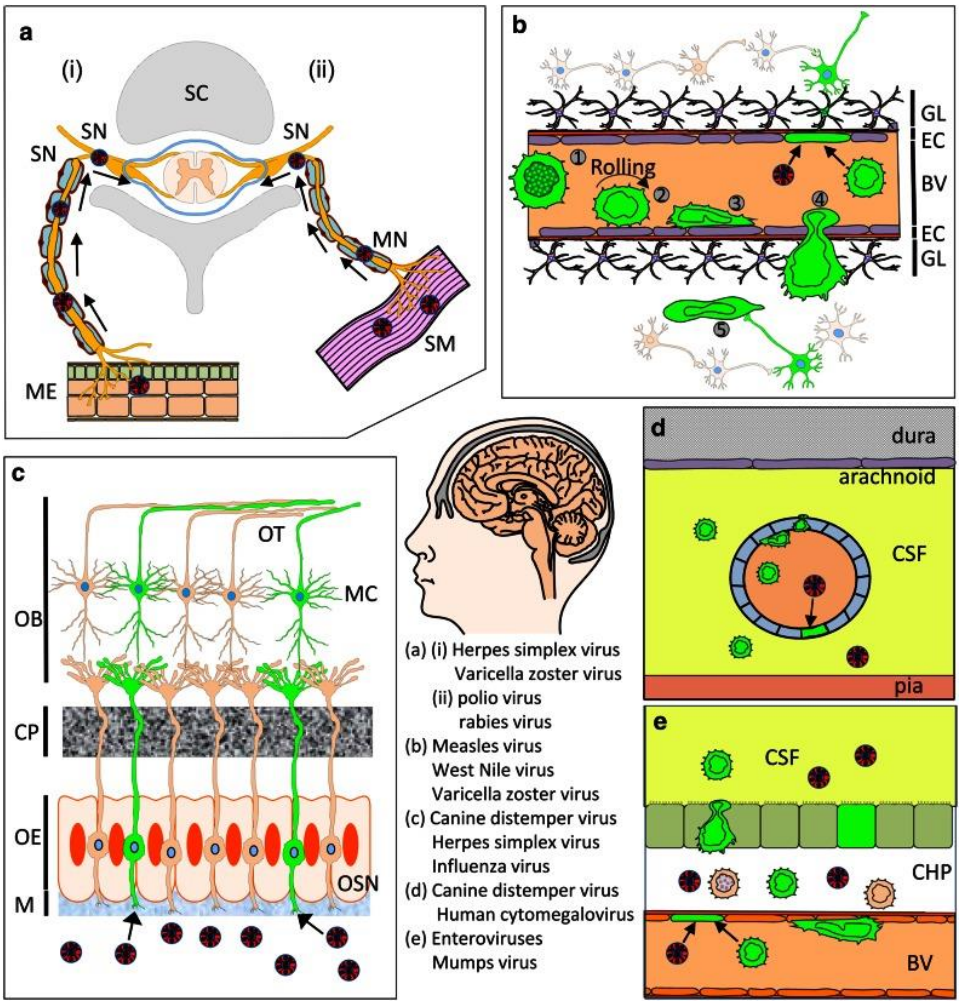
Foley JH, Conway EM.
Cross Talk Pathways Between Coagulation and Inflammation.
Circ Res. 2016 Apr 29;118(9):1392-408. doi: 10.1161/CIRCRESAHA.116.306853.
https://www.ahajournals.org/doi/full/10.1161/CIRCRESAHA.116.306853

[42] Pereira A.
Long-Term Neurological Threats of COVID-19: A Call to Update the Thinking About the Outcomes of the Coronavirus Pandemic.
Front Neurol. 2020;11:308. Published 2020 Apr 17. doi:10.3389/fneur.2020.00308
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7182030/

Some viruses possess tropism for nerve tissue and are therefore classified as neurotropic (e.g., herpes simplex virus type 1, rabies virus).

These **viruses enter the brain** through various pathways, including retrograde axonal transport along axons, hematogenous spread across the blood-brain barrier (BBB), the blood-cerebrospinal fluid barrier, the meningeal-cerebrospinal fluid barrier and by direct infection of endothelial cells or spread of infected leukocytes to the brain through the BBB.

Once in the brain, these viruses disrupt the complex organization of neural circuits either directly leading to **neuronal damage** or indirectly through host immune response pathways, causing neuropathologies and **immediate** or **delayed neurological manifestations** [43].



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4713712/

Routes of virus spread into the central nervous system. **a** Infection of peripheral nerves. (*i*) Virus spread from mucosal epithelium (*ME*) to sensory and autonomic neurons (*SN*) following infection of axon termini. Retrograde axonal transport results in virus spread to the spinal cord (*SC*). (*ii*) Virus infection of motor neurons (*MN*) at neuromuscular junctions in smooth muscle (*SM*) results in retrograde axonal transport to the spinal cord and the brain. **b** Blood–brain barrier (BBB). Virus-infected lymphocytes (*green*) (*1*) in blood vessels (*BV*) 'roll' along the endothelium (*2*), attach to the endothelial cells (*3*) and transverse the endothelial cell layer (*EC*) (*4*) and the glia limitans (*GL*). Virus spread to neurons (*5*) is assumed to occur following contacts with uninfected neurons. Alternatively, direct virus infection of endothelial cells may occur with subsequent spread into the brain parenchyma resulting in neuronal infection. **c** Infection of olfactory neurons. Virus present in the mucosa (*M*) of the upper respiratory tract can directly infect olfactory sensory neurons (*OSN*) present in olfactory epithelium (*OE*). Anterograde axonal transport leads to spread of virus within axonal bundles passing through the cribriform plate (*CP*) into the olfactory bulb (*OB*). Trans-synaptic spread to mitral cells (*MC*) results in virus spread along the olfactory tract (*OT*) to other brain regions. **d** Meningeal blood–cerebrospinal fluid (*CSF*) barrier. Virus-infected leukocytes in meningeal blood vessels present within the sub-arachnoid space between the pia and arachnoid roll, attach to the endothelium and transverse endothelial cells into the CSF. Direct infection of endothelial cells may also lead to virus spread into the CSF. **e** Blood–cerebrospinal fluid barrier. Virus-infected leukocytes or cell-free virus present within blood vessels of the choroid plexus (*CHP*) transverse the endothelium as described previously in **b**, **d**. This can lead to infection of epithelial cells and apical release of virus or spread of virus-infected leukocytes across the CHP epithelium into the CSF.

[43] Ludlow M, Kortekaas J, Herden C, et al.
Neurotropic virus infections as the cause of immediate and delayed neuropathology.
Acta Neuropathol. 2016;131(2):159-184. doi:10.1007/s00401-015-1511-3
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In the short term, neurotropic viral infections can cause inflammation of the brain parenchyma and lead to **encephalitis** or brain-targeted **autoimmune responses** in predisposed individuals [44].
Possible long-term effects on hosts may include alterations in emotional and cognitive behavior, as shown in experimental animals through persistent alterations in the expression of genes involved in the regulation of synaptic activities in key brain areas [45].
Axonal transport of neurotropic viruses can also transform intrinsically disordered proteins, such as α-synuclein (α-syn), into promiscuous ligands that can form toxic aggregates and travel along neuronal pathways from postganglionic enteric neurons to central nervous system nerves and cause **cell death in various areas of the brain**. (Braak Hypothesis) [46]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6558190/
The intestinal epithelium is a multifunctional interface. Bidirectional interaction between the brain and the gut is mediated by neural pathways, such as the vagus nerve (VN pathway), and humoral pathways, such as lymphatic tissue and blood flow (non-VN pathway). A monolayer of epithelial cells separates the intestinal lumen and the complex intestinal microbiome from the underlying enteric lymphoid and nerve tissues. The structure of alpha-synuclein amyloid fibrils (PDB 2N0A) is based on atomic resolution molecular data from NGL Viewer [47]. Members of the gut microbiome and their extracellular compounds may trigger responses in the VN through enteroendocrine cells, which are contacted by vagus nerve terminals through specialized structures called neuropods (NP) [48]. Microbial antigens can cross the intestinal epithelium via microfold cells, playing a central role in localized inflammatory responses [49]. Toll-like receptors are microbiome-sensing proteins, present in intestinal epithelial cells, that mediate the recognition of commensal bacteria from harmful/inflammatory ones. ENS, enteric nervous system; M, microfold cells; NP, neuropods; PP, Peyer's patches; TLR4, Toll-like receptor 4; VN, vagus nerve.

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Viral-induced suppression of self-reactive T cells: Lessons from neurotropic coronavirus-induced demyelination.
J Neuroimmunol. 2017 Jul 15;308:12-16. doi: 10.1016/j.jneuroim.2017.01.003. Epub 2017 Jan 11.
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https://www.nature.com/articles/4001545

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Sharma S, Awasthi A, Singh S.
Altered gut microbiota and intestinal permeability in Parkinson's disease: Pathological highlight to management.
Neurosci Lett. 2019 Nov 1;712:134516. doi: 10.1016/j.neulet.2019.134516. Epub 2019 Sep 24. PMID: 31560998.
https://www.e-jmd.org/journal/view.php?number=246

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Bioinformatics. 2018 Nov 1;34(21):3755-3758. doi: 10.1093/bioinformatics/bty419.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6198858/

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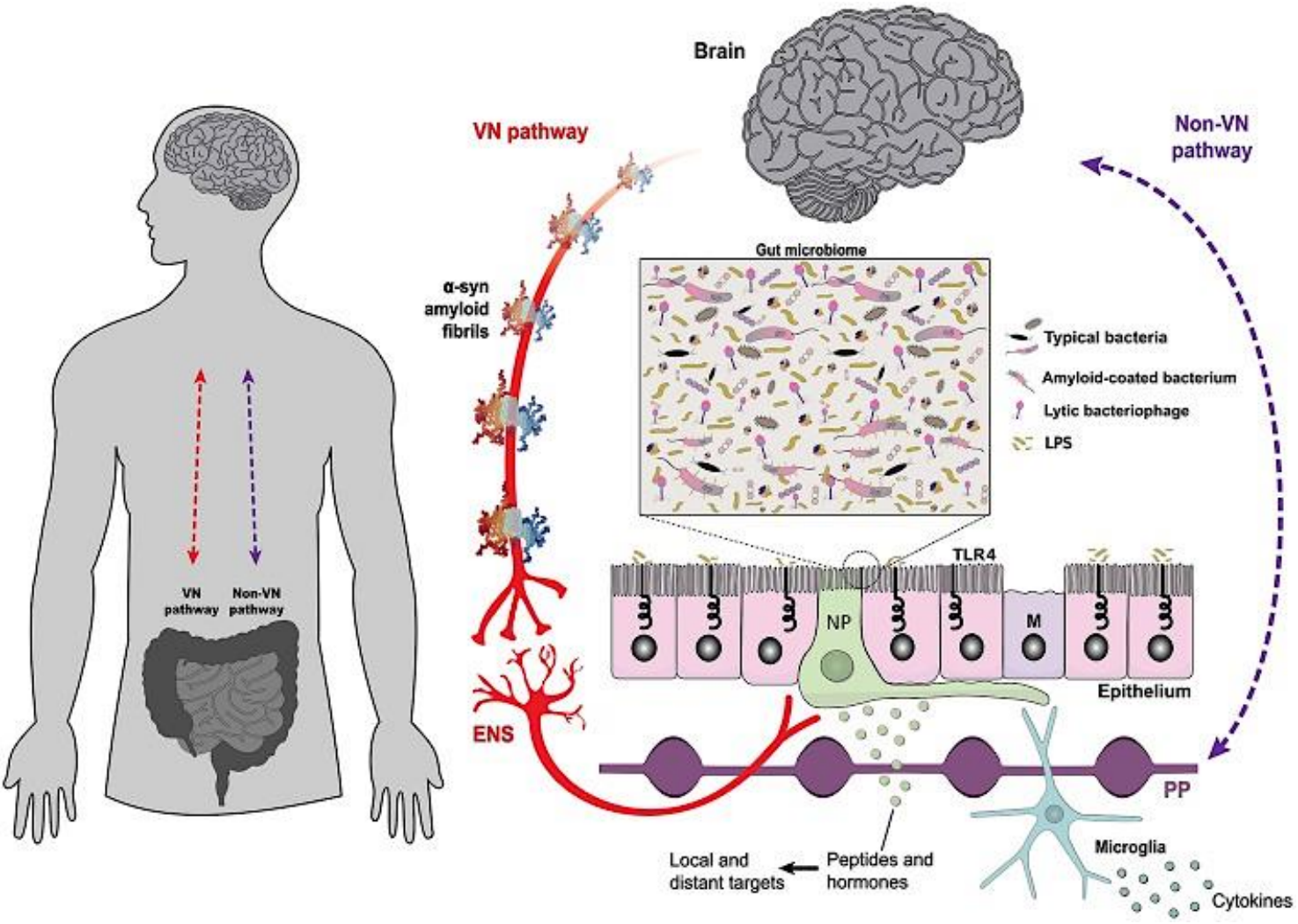

While the most common symptoms from the early stage of COVID-19 disease include fever, fatigue, dry cough, myalgia, and dyspnea, other less common symptoms include headache, abdominal pain, diarrhea, nausea, and vomiting [50]. In addition, it has been found that most patients also complain of an impairment of both olfactory and gustatory perception [51] and these are considered early markers of COVID-19 infection.[52]

[50] https://www.ecdc.europa.eu/en/covid-19/latest-evidence/clinical

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Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China.
JAMA. 2020 Mar 17;323(11):1061-1069. doi: 10.1001/jama.2020.1585.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7042881/

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COVID 19-Induced Smell and Taste Impairments: Putative Impact on Physiology.
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Am J Otolaryngol. 2021 Jan 6;42(2):102889. doi: 10.1016/j.amjoto.2020.102889.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7833280/

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Systematic Review and Meta-analysis of Smell and Taste Disorders in COVID-19.
OTO Open. 2020 Sep 11;4(3):2473974X20957975. doi: 10.1177/2473974X20957975.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7488903/

Although there is long-standing evidence that human coronaviruses [53], such as SARS-CoV-2 [54], can spread to the brain from the respiratory tract, the occurrence of gastrointestinal symptoms [55] **suggests that the gastrointestinal system is a possible route of invasion and transmission to the enteric nervous system** (ENS). [56]
While the effects of COVID-19 on olfactory and gustatory perception may be transient, the **possibility of SARS-Cov-2** [57]

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Neurologic Alterations Due to Respiratory Virus Infections. Front Cell Neurosci. 2018 Oct 26;12:386. doi: 10.3389/fncel.2018.00386.
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Human Coronaviruses and Other Respiratory Viruses: Underestimated Opportunistic Pathogens of the Central Nervous System?
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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7385206/

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iScience. 2020 Dec 18;23(12):101839. doi: 10.1016/j.isci.2020.101839. Epub 2020 Nov 25
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7685946/

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Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia.
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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7313997/

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image, in which infectious agents (viruses, bacteria, prions) and vaccine toxic substances can lead to the activation of microglia, the macrophage component of the central nervous system, and the establishment of a vicious inflammatory cycle that leads to degeneration of the nervous tissue in which it is triggered.

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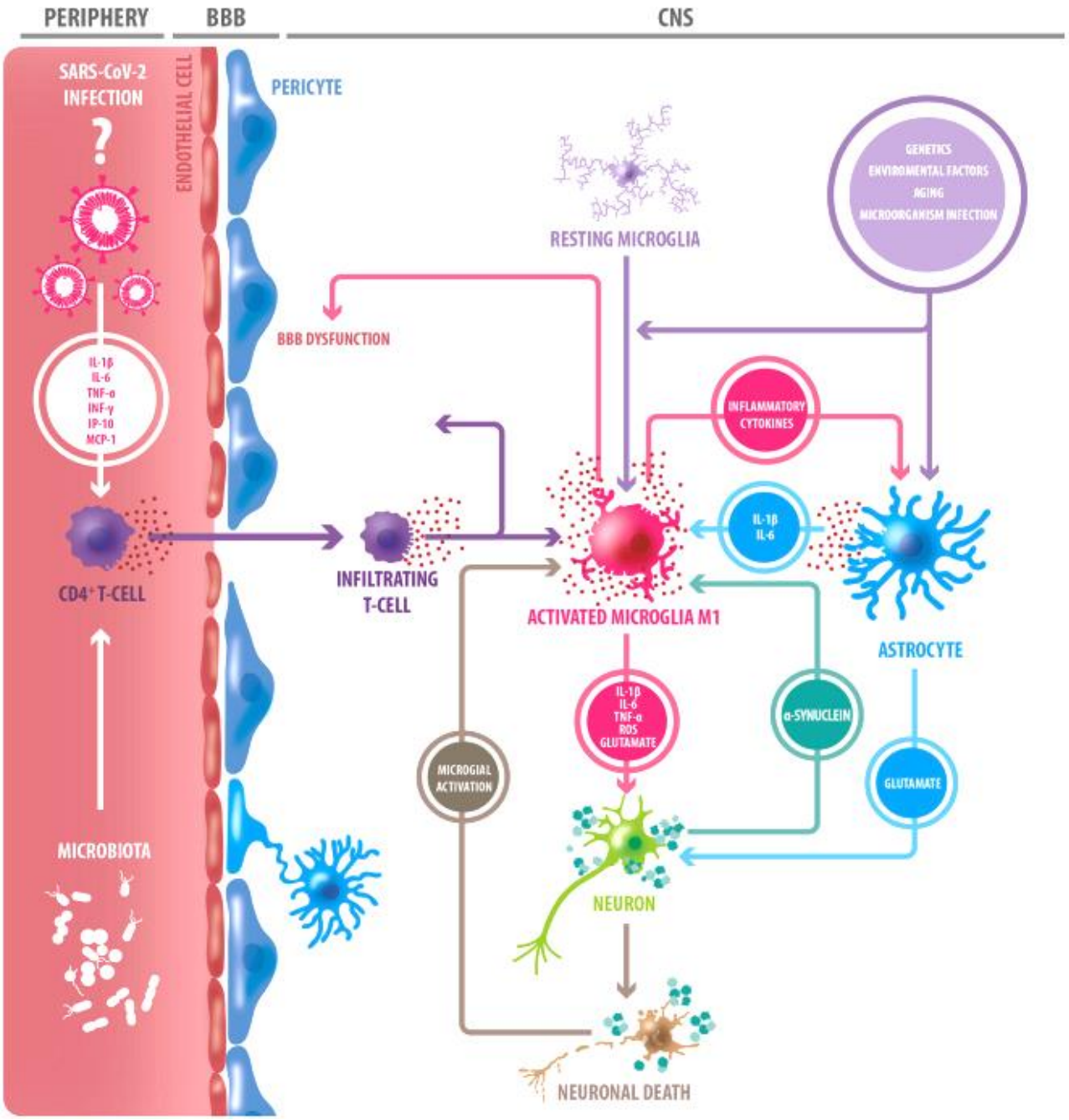

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Triggering factors of the neuroinflammatory process. Aging, in addition to genetic and environmental factors, and infections of certain microorganisms, can trigger a neuroinflammatory response through microglial and oligodendroglial activation. Activated microglia adopt an M1 inflammatory phenotype, secreting proinflammatory cytokines, reactive oxygen species (ROS), and glutamate; factors that cause neuronal damage. In this context, astrocytes become reactive, and like microglia, they secrete proinflammatory cytokines. Many of these cytokines act on microglial cells, exacerbating microglial activation, and favoring neuronal damage. The release of TNF-alpha by microglia induces increased glutamate release by astrocytes: a detrimental event for neurons. In this context, degenerating and/or dead neurons are observed, which in turn trigger microglial activation. Protein accumulation (e.g., alpha-synuclein) is another triggering factor for microglial activation. Microglia degrades and presents components of dead cells and protein aggregates to CD4+ T lymphocytes. This, in conjunction with the release of cytokines, results in the infiltration of CD4 + T cells, which release more proinflammatory cytokines, leading to greater neurodegeneration. As a consequence of this neuroinflammation, the blood-brain barrier (BBB) becomes dysfunctional, leading to the entry of peripheral immune cells. In the periphery, gut microbiota can trigger inflammation mediated by innate immune cells. The SARS-CoV-2 virus generates a “cytokine storm” at the peripheral level, therefore, it could have a similar effect. Inflammatory cytokines from peripheral blood circulation could also contribute to BBB permeabilization.

Parkinson's disease (PD) is a common neurodegenerative disorder associated with the progressive loss of dopaminergic neurons located in the nucleus of the substantia nigra pars compacta (SNpc) of the midbrain due to the

accumulation of α-synuclein (α-syn) aggregates. **Interestingly, the prodromal or preclinical phase of PD is also characterized by olfactory and gastrointestinal symptoms** [60].

The hypothesis for the etiology of sporadic Parkinson's disease (PD) proposes that a neurotropic virus invading nerve tissue through the nasal cavity and gastrointestinal tract induces α-syn to transform into a prion-like ligand and be transmitted in key areas such as the SNpc [61]. Many studies suggest that intestinal toxins can induce the formation of α-syn aggregates in the ENS, which can then be transmitted as prions to the CNS via the VN.[62]

Evidence of the role played by toxins in inducing parkinsonism and the relative paucity of familiarity (approximately 10%) [63] emphasize the importance of environmental and lifestyle factors over genetic factors in the etiology of the disease [64].

Some chronic diseases have been associated with a phenomenon called evolutionary maladjustment when ancestral traits are no longer adaptive in modern contexts. [65]

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It is important to note that the PD [70] and Creutzfeldt-Jakob prion disease [71] have been reported in the literature as diseases caused by COVID-19, and PD as a possible adverse reaction from mRNA vaccine, [72] supporting a role for a

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neuroinflammatory process induced by viral infection [73] and prion-like aggregate formation in the onset of these diseases.

In this regard, SARS-Cov-2 has prion-like sequences in the receptor binding domain of the S1 region of the spike protein, a unique feature compared with other coronaviruses, that increase viral binding to its ACE2 receptor and thus play a major functional role in virulence. **[74] It is unknown whether these sequences can lead to the formation of pathological prion spike proteins.**

In addition, in a recent article, **Dr. J.B. Classen** [75] conducted a bioinformatics **analysis to identify the possible presence of sequences in the mRNA of the "Pfizer" vaccine that could activate TDP-43 and FUS, two proteins with prion-like properties that bind RNA and consequently are able to induce prion diseases** [76].
From this preliminary analysis it appears that the vaccine mRNA contains sequences that are thought to induce TDP-43 and FUS to aggregate in their prion-like structure.
In particular, it has been shown that the GGUA RNA sequences [77], UG-rich sequences [78], repetitions of tandem UG [79] and quadruplex sequences G [80], have a higher affinity for binding TDP-43 and/or FUS and can induce TDP-43 or FUS to acquire their pathological configurations in the cytoplasm. In the analysis done, a total of sixteen tandem UG repeats (ΨGΨG), additional UG-rich sequences (ΨG), and two GGΨA sequences were identified.
The hypothesis is that vaccine mRNA may potentially act as a cofactor in the formation of pathological prion-like proteins. The cellular receptor for SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2), which plays a role in the metabolism of angiotensin peptides involved in the control of vasoconstriction and blood pressure [81]. ACE2 is found in several tissues associated with cardiovascular function, but also in the brain, including brainstem nuclei involved in

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cardiorespiratory regulation [82]. Therefore, respiratory problems in patients with COVID-19 could also result from the direct action of SARS-CoV-2 in the respiratory control nuclei in the brain [83].

Through its binding to ACE2 receptors, SARS-CoV-2 can spread transneuronally to distant brain targets, similar to other neurotropic viruses [84], as predicted by Braak's hypothesis. **It follows that although recovery from the acute phase of infections is certainly a relief in terms of public health, as it helps to stop the spread of infection, the long-term neurological and multi-organ effects of the disease even in asymptomatic healthy individuals could become a serious health problem to manage and must be taken into due consideration.**
Indeed, COVID-19 can lead to sequelae and other medical complications that last weeks or months after initial recovery, which have been referred to as Long-COVID **or long-term COVID.** [85]
Up to 30-50% of COVID-19 survivors experience persistent dyspnea and cough for 2-3 months. Chest pain, heart palpitations, and tachycardia are also common symptoms of long-COVID that occur in approximately 20-40% of survivors.
Long-COVID also involves a myriad of neurological symptoms that can occur in 20-70% of cases, such as fatigue, myalgia, insomnia, headache, depression, anxiety, alterations in smell and taste, and cognitive impairment. As the invasion of SARS-CoV-2 into the brainstem can disrupt neurotransmitter systems in the brain, causing various neurological symptoms.
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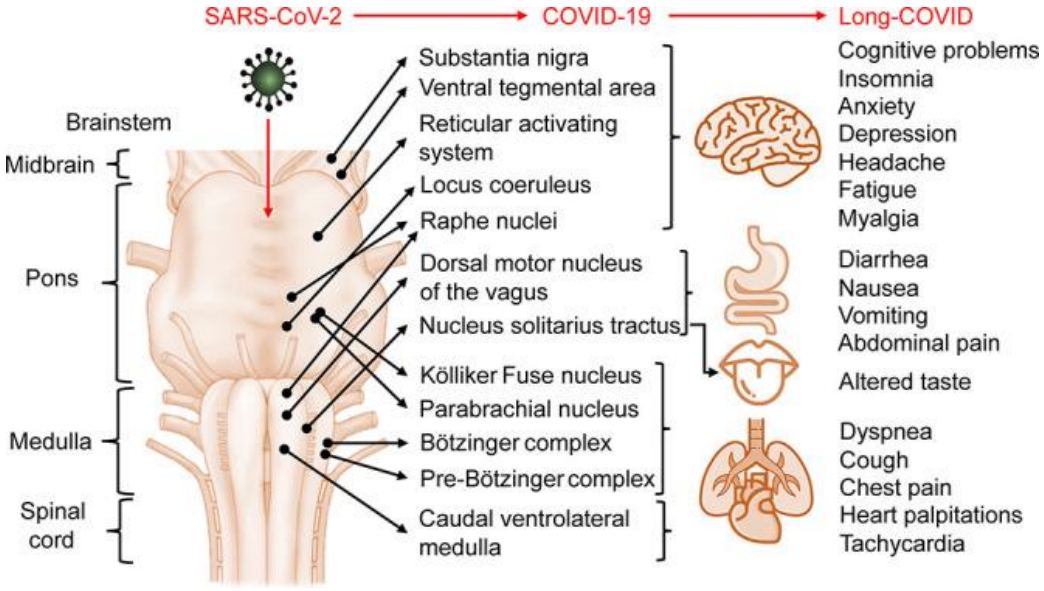
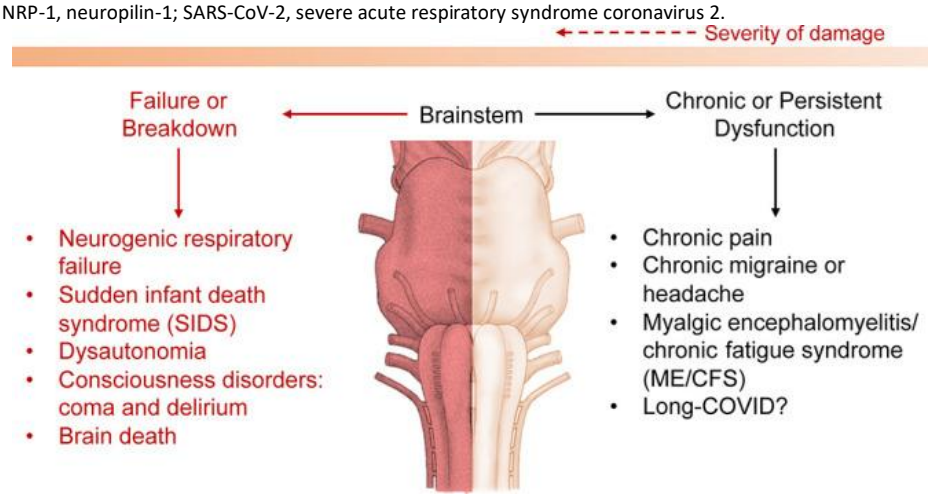
Importantly, brain imaging has found that the severity of ME/CFS symptoms correlates with brainstem dysfunction, particularly at the RAS. [87] Thus, brainstem dysfunction can result in fatal or persistent disease, of which the latter may include long-COVID.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7874499/
Overview of the brainstem dysfunction hypothesis in long-COVID. Note that nuclei and subparts of the brainstem's medulla, pons, and midbrain are not drawn to scale and may not reflect the exact neuroanatomical structures. Abbreviations used are the following: ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; NRP-1, neuropilin-1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7874499/
Overview of the disorders related to brainstem dysfunction, which can be fatal or persistent. Note that darker shades of red/orange indicate more severe conditions.

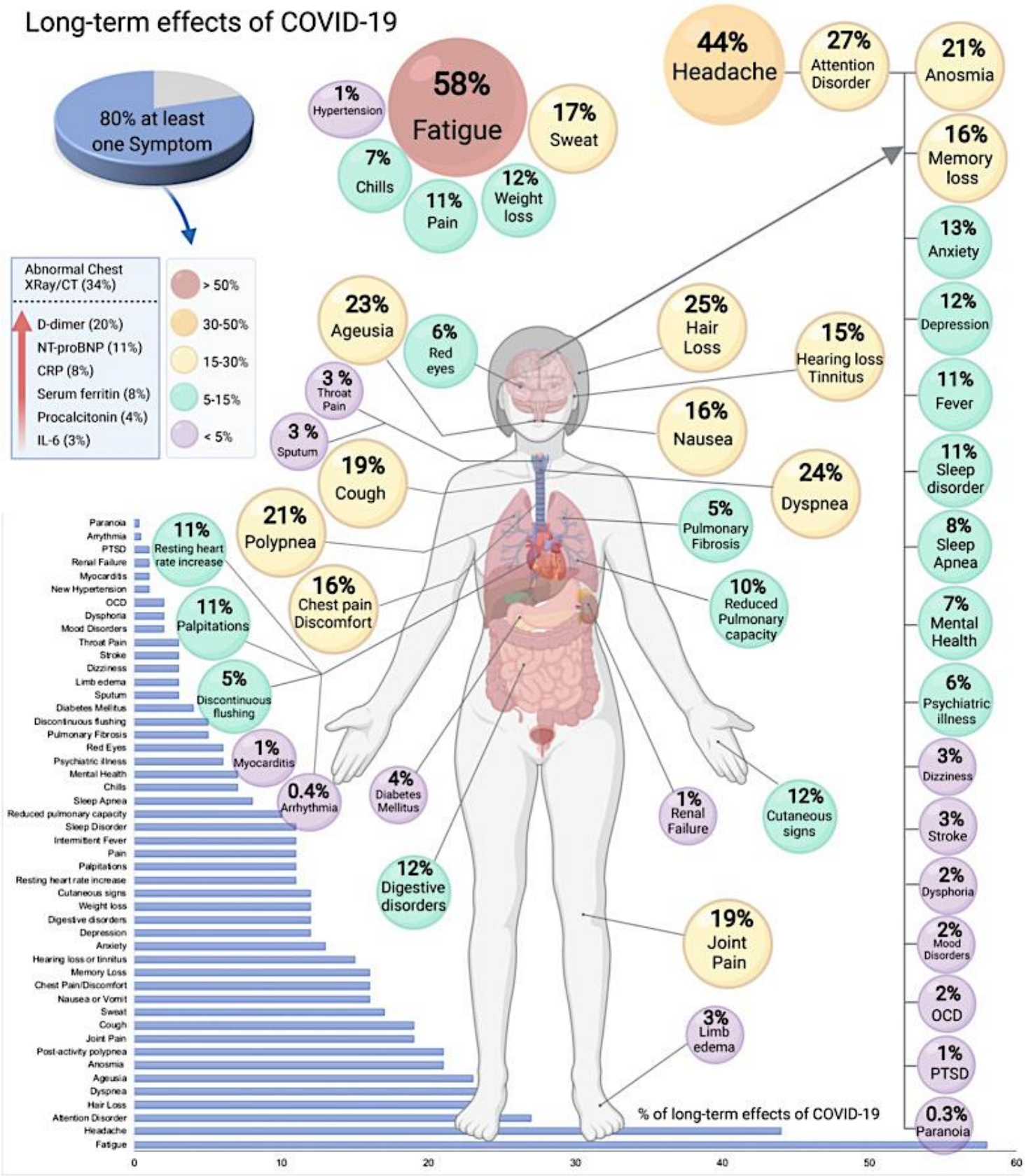
In addition to neurological damage, diseases such as stroke and diabetes mellitus were also found.

[87] VanElzakker MB, Brumfield SA, Lara Mejia PS.
Neuroinflammation and Cytokines in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): A Critical Review of Research Methods [published correction appears in Front Neurol. 2019 Apr 02;10:316] [published correction appears in Front Neurol. 2020 Sep 17;11:863]. Front Neurol. 2019;9:1033. Published 2019 Jan 10. doi:10.3389/fneur.2018.01033
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6335565/

Barnden LR, Shan ZY, Staines DR, et al.
Hyperintense sensorimotor T1 spin echo MRI is associated with brainstem abnormality in chronic fatigue syndrome.
Neuroimage Clin. 2018;20:102-109. doi:10.1016/j.nicl.2018.07.011
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6309570/

Laboratory parameters measured included elevated values of interleukin-6 (IL-6), procalcitonin, serum ferritin, C-reactive protein (CRP), N-terminal hormone (NT)-pro BNP and D -dimer. In addition, abnormal computed tomography/computed tomography of the chest was also identified.

Given the similarity between the mechanisms of COVID-19 damage and vaccine adverse reactions (see "Pfizer" paper below), it is conceivable that many of the symptoms and pathologies associated with long-COVID may also be present as long-term consequences of vaccination.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7852236/
Long-term effects of coronavirus disease 2019 (COVID-19).
The meta-analysis of the studies included an estimate for one symptom or more reported that 80% of the patients with COVID-19 have long-term symptoms.
Abbreviations: C-reactive protein (CRP), computed tomography (CT), Interleukin-6 (IL-6), N-terminal (NT)-pro hormone BNP (NT-proBNP), Obsessive Compulsive Disorder (OCD), Post-traumatic stress disorder (PTSD).

The following are the adverse reactions collected during the pharmacovigilance of the Pfizer vaccine. As can be seen, most of the pathologies encountered can be explained by the mechanisms of induction injury discussed above. [88]

COVID-19 mRNA Pfizer- BioNTech vaccine analysis print
Report Run Date: 12-Feb-2021
Data Lock Date: 11-Feb-2021 19:00:03
All UK spontaneous reports received between 09/12/20 and 07/02/21 for mRNA Pfizer/BioNTech vaccine analysis print

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/962405/COVID-19_mRNA_Pfizer-_BioNTech_Vaccine_Analysis_Print.pdf

INTEGRATION IN THE DNA

New biotechnologies used to produce COVID-19 vaccines have raised important questions about their safety. One of the concerns expressed relates to the potential risk of mRNA integration into human DNA.
It should be noted that **while there is a theoretical concern for integration into the host genome with regard to plasmid DNA vaccines, this concern is not shared for mRNA-based vaccines for the following reasons.**
(1) mRNA remains in the cytoplasm and is not transported into the nucleus.

(2 For integration, the mRNA should be converted into a DNA molecule. Although it is possible for single-stranded DNA to be integrated, the form of DNA integration is generally double-stranded DNA. This requires the presence of a reverse transcriptase and appropriate primers and complementary binding sites on the mRNA to first generate single-stranded DNA and then convert this single-stranded DNA to double-stranded DNA, which still requires appropriate primers and binding sites. In retroviruses, this process occurs in the retrovirus particle. This is not the case with mRNA vaccines, because while endogenous reverse transcriptase is present in mammalian cells, the enzymes and RNA are not in the appropriate complex to allow efficient reverse transcription.

(3) The final step of retroviral integration requires the activity of viral integrase, which is still located in the retroviral particle. It has been shown that integration of the naked DNA double helix is very inefficient.

(4) Vaccine mRNA has been shown to degrade in a relatively short time once absorbed into body cells. Finally, because cellular mRNA is more abundant than vaccine mRNA, it is highly unlikely that a cellular reverse transcriptase (RT) would preferentially copy vaccine mRNA over cellular mRNA. For all these reasons, the risk of integration of mRNA vaccines has been considered negligible. [89]

[88] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/962405/COVID-19_mRNA_Pfizer-_BioNTech_Vaccine_Analysis_Print.pdf

[89] Knezevic I, Liu MA, Peden K, Zhou T, Kang HN.
Development of mRNA Vaccines: Scientific and Regulatory Issues.
Vaccines (Basel). 2021 Jan 23;9(2):81. doi: 10.3390/vaccines9020081.
https://www.mdpi.com/2076-393X/9/2/81