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ZEROSPIKE PROJECT
- Short summary on the Spike Protein -

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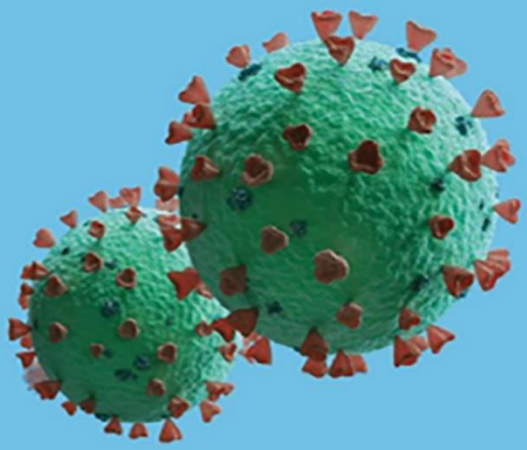

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ZEROSPIKE PROJECT

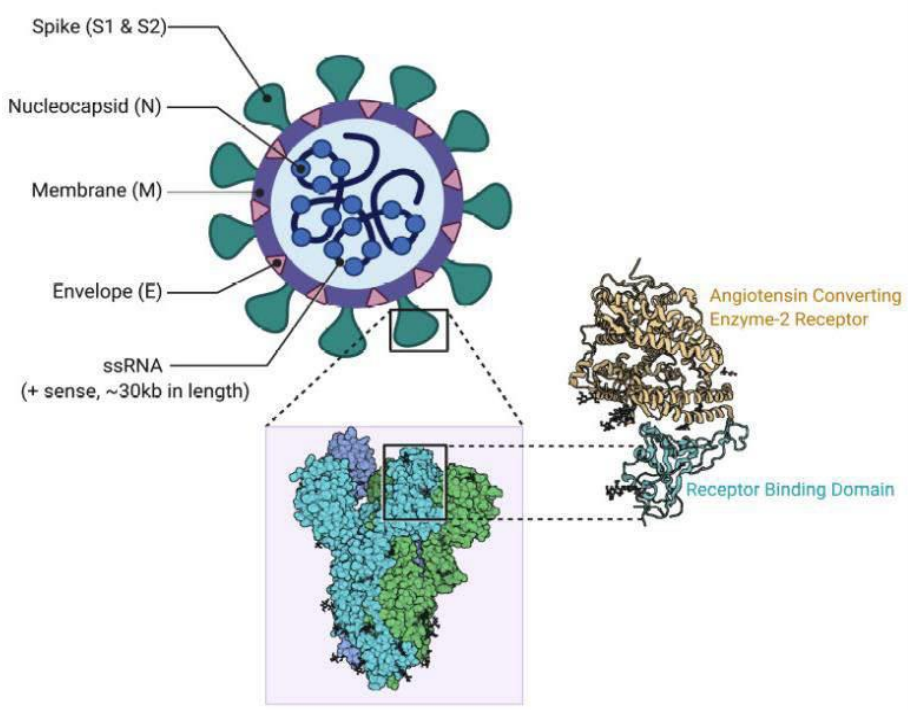
The Zerospike research project was started with the aim of studying the toxicological characteristics of the Spike protein present both in the SARS-Cov-2 virus and produced by human bodies injected with the new Covid 'vaccines'. A further aim of the ZeroSpike project was to find useful remedies to detoxify the body of this dangerous Spike protein in cases of acute Covid, long Covid and in cases of adverse effects of the Covid 'vaccine'.

THE CHARACTERISTICS OF SARS-COV-2 AND SPIKE PROTEIN

Coronaviruses are important human and animal pathogens. In late 2019, a new coronavirus was identified as the cause of a cluster of pneumonia cases in Wuhan, a city in China's Hubei Province, which spread rapidly, causing an epidemic throughout China, followed by a global pandemic. In February 2020, the World Health Organization named the disease COVID-19, which stands for "coronavirus disease 2019." The virus that causes COVID-19 was initially referred to as 2019-nCoV and later named "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) because of its similarity to the SARS-Cov-1 virus responsible for a 2003 outbreak in China.[1]

The genome of SARS-CoV-2 is a single-stranded positive-sense strand that encodes for nonstructural proteins (NSPs, such as 3-chymotrypsin-like protease, papain-like protease, helicase and RNA-dependent RNA polymerase), structural proteins and accessory proteins.
SARS-CoV-2 has four structural proteins: spike protein (S), envelope protein (E), membrane protein (M) and nucleocapsid protein (N). Among these proteins, the trimeric S protein is essential for virus-cell-receptor interactions during viral entry.

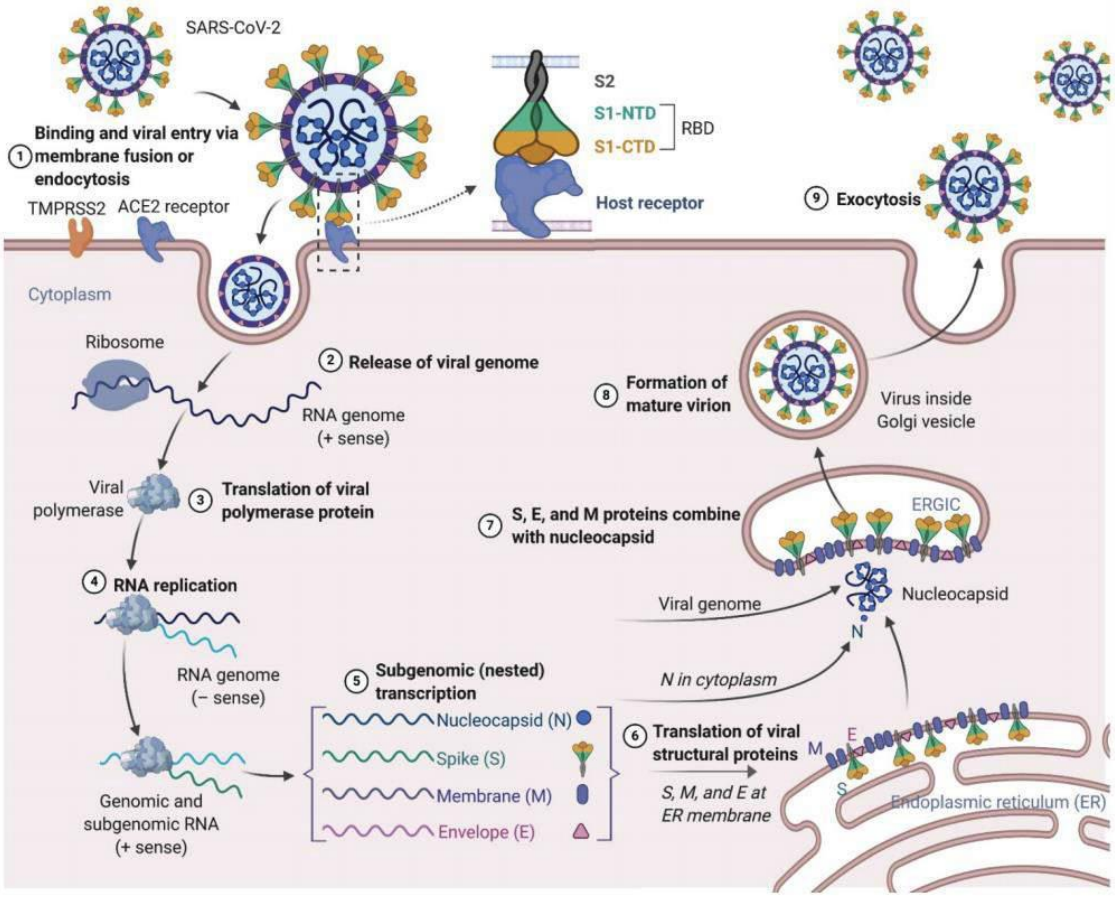
[1] https://www.uptodate.com/contents/covid-19-epidemiology-virology-and-prevention



https://www.ncbi.nlm.nih.gov/books/NBK554776/

The S protein comprises an S1 N-terminal subunit responsible for receptor-virus binding and an S2 C-terminal subunit responsible for cell membrane-virus fusion.
S1 is further subdivided into an N-terminal domain (NTD) and a receptor-binding domain (RBD).
SARS-CoV-2 enters cells through the S protein, which primarily binds to the human angiotensin-converting enzyme 2 (ACE2) receptor and employs the cellular serine protease TMPRSS2 for S protein activation.
This binding triggers a cascade of events leading to fusion between cell and viral membranes for virus entry into cells. The viral RNA genome is released into the cytoplasm after membrane fusion.
Polyproteins are subsequently synthesized to encode the viral replicase-transcriptase complex and Viral RNA is then synthesized by RNA-dependent RNA polymerase.
Structural protein synthesis is followed by the assembly and release of viral particles.
These stages of the viral life cycle provide potential targets for vaccines and therapies to prevent and treat SARS-CoV-2 infection.[2]

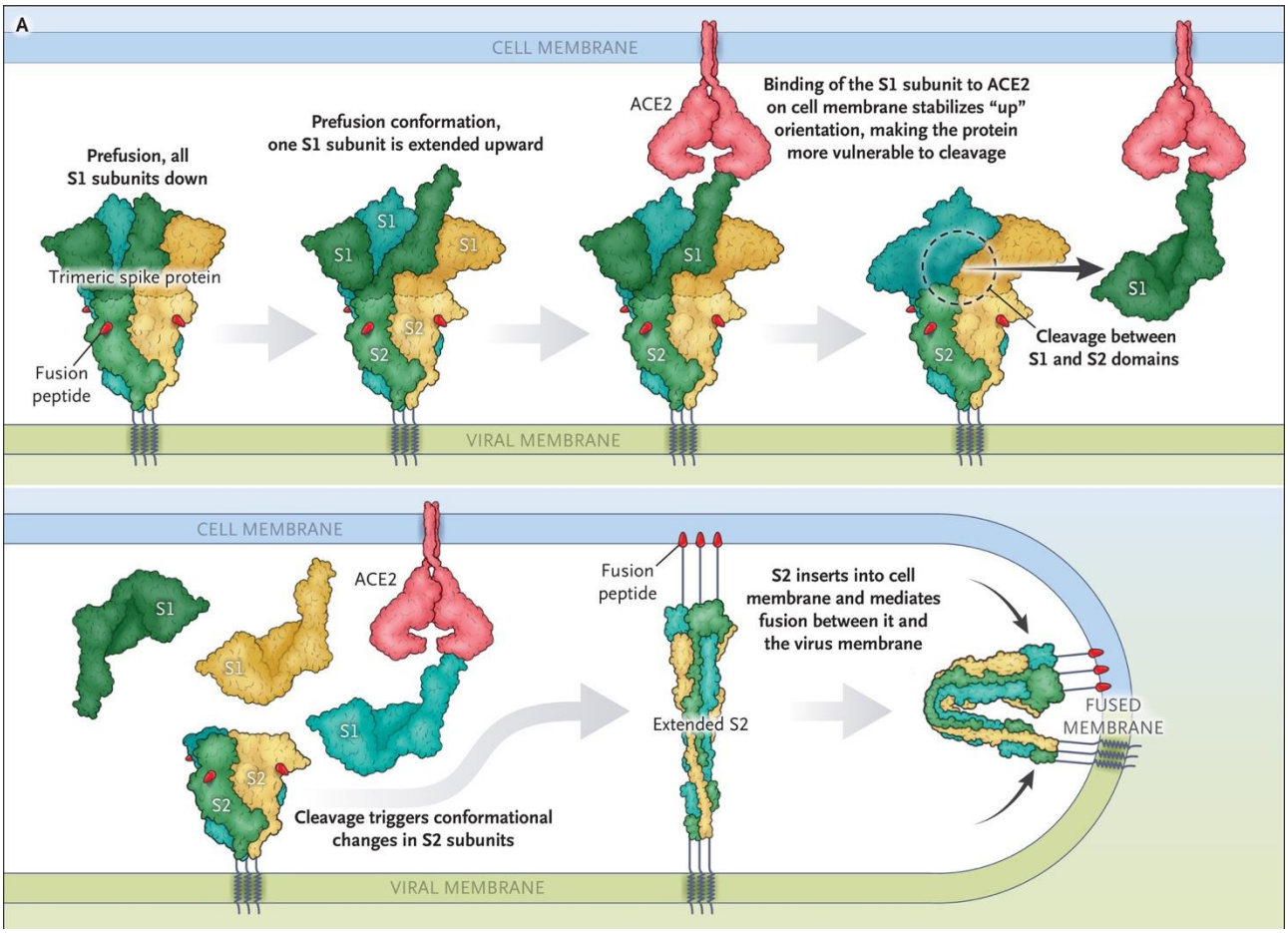
[2] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8447893/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7359073/

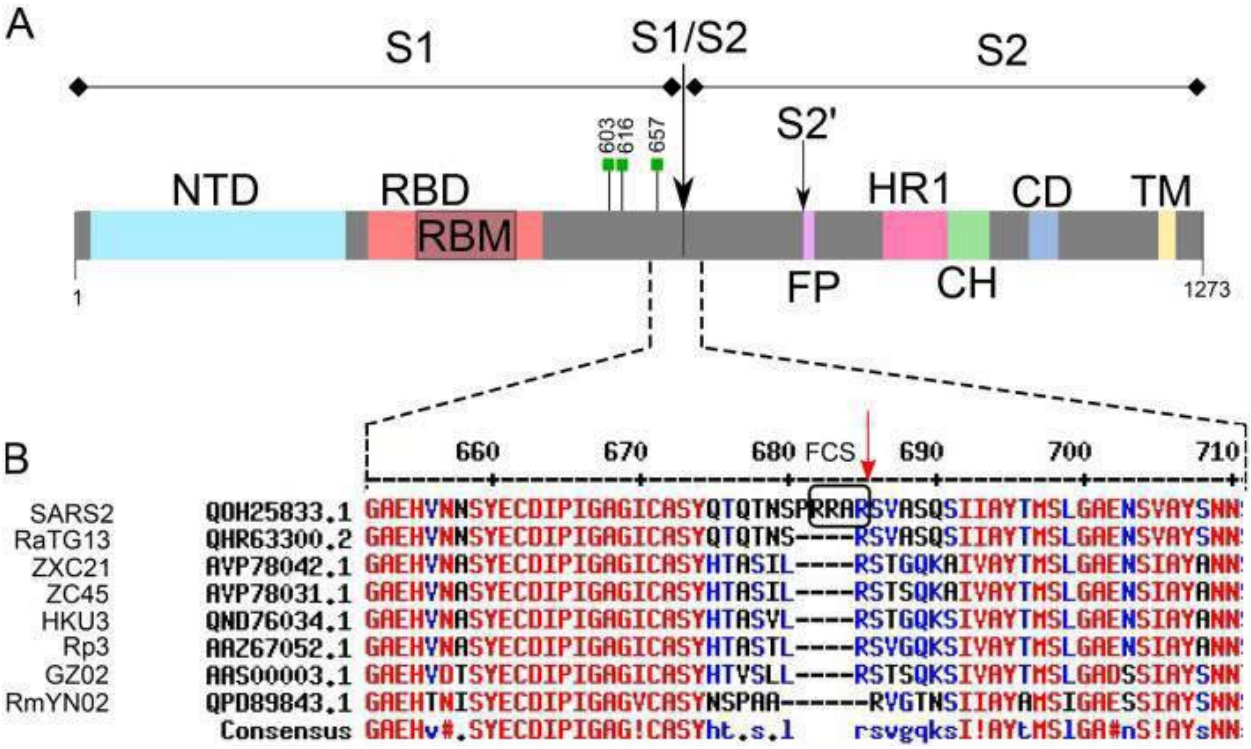
The S protein is a trimeric class I fusion protein that exists in a metastable prefusion conformation that undergoes a substantial structural rearrangement to fuse the viral membrane with the host cell membrane. The prefusion state is referred to as the "down" conformation and is that of inaccessibility to the ACE receptor. When the S1 subunit binds to the receptor, it performs a hinge-like conformational movement, which allows it to expose the other RBDs in a so-called "up" conformation and causes the S2 subunit to assume a postfusion conformation that leads the viral membrane to fuse with the cell membrane.[3]

[3] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7474903/



It is interesting that SARS-CoV-2 has an insertion of four amino acids (PRRAR polybasic site) at the S1 and S2 junction as compared with the S protein of SARS-CoV-1, in which it is absent.
These four additional amino acids constitute the cutting site for a specific human protease called furin. Given the virtually ubiquitous expression of furin-like proteases, this feature is responsible for the wider cellular and tissue tropism of SARS-CoV-2 compared with SARS-CoV-1, as well as the increase in its transmissibility and pathogenicity.
It has recently been shown that, like TMPRSS2, furin is essential for SARS-CoV-2 entry into host cells. Specifically, SARS-CoV-2 entry requires sequential cleavage of the spike glycoprotein at S1/S2 and S2' cleavage sites to mediate membrane fusion.[4]
In addition, the "RRAR" motif creates a C-terminal motif (CendR) with a binding site for neuropilin membrane receptors (NRP1 and NRP2), which are more widely expressed than ACE2.
Neuropilin-1 (NRP1) shows high expression in the respiratory and olfactory epithelium, and may be implicated in the neurological manifestations of COVID-19 by promoting the entry of SARS- CoV-2 into the brain through the olfactory epithelium.[5]

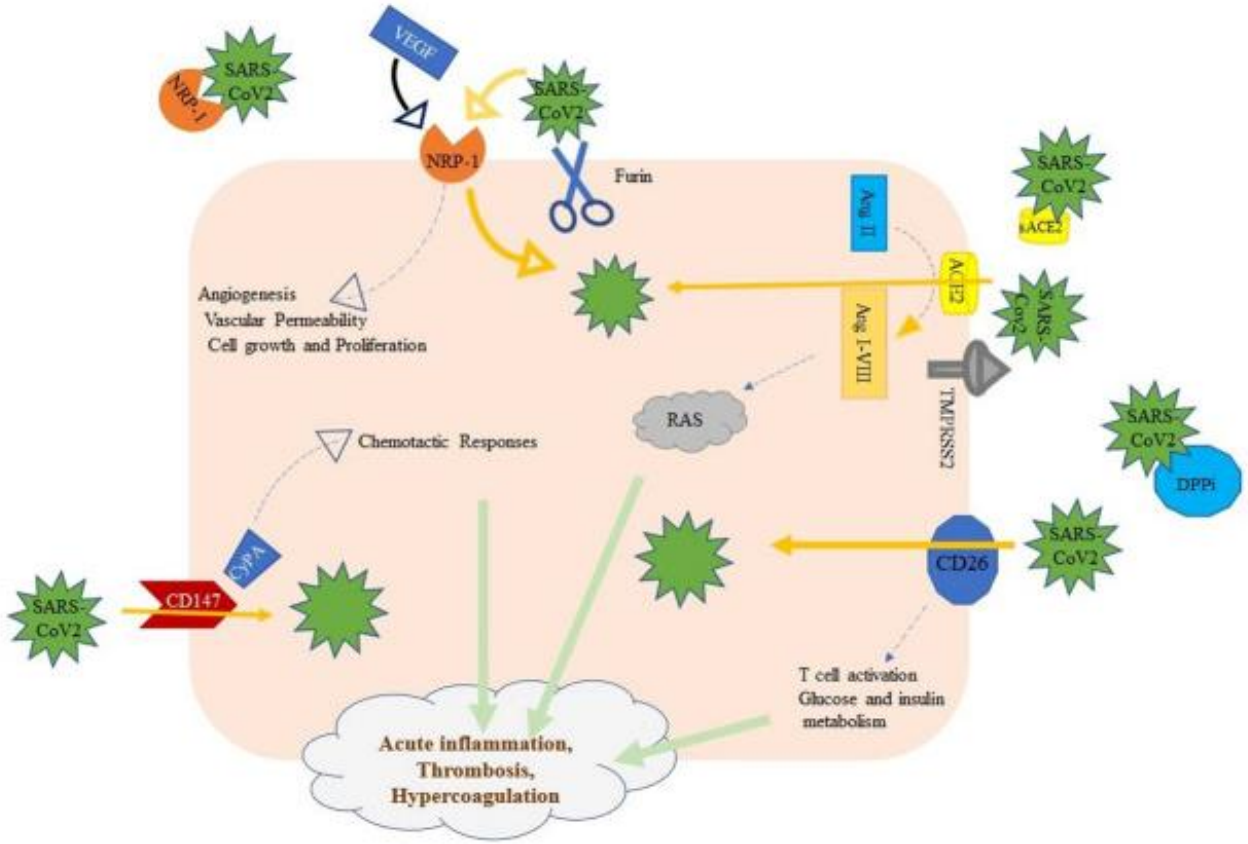
[4] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9044946/
[5] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7857391/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7993900/

Although ACE2 plays a key role in SARS-CoV-2 replication, its expression profiles are not fully associated with infection patterns, immune responses, and clinical manifestations. In addition, SARS-CoV-2 infects cells lacking ACE2 and the infection is resistant to monoclonal antibodies against the spike RBD in vitro, indicating that some human cells possess alternative ACE2-independent receptors, which may mediate SARS-CoV-2 entry. These receptors, in addition to NRP1 mentioned above, include CD147, AXL, CD209L/L-SIGN/CLEC4M, CD209/DC-SIGN/CLEC4L, CLEC4G/LSECtin, ASGR1/CLEC4H1, LDLRAD3, TMEM30A and KREMEN1. Most of these receptors are known to be involved in the entry of other viruses and to modulate cellular functions and immune responses. The SARS-CoV-2 omicron variant shows altered cellular tropism and an associated change in the cellular entry pathway, indicating that the emerging variants may use alternative receptors to escape the immune pressure against ACE2-dependent viral entry caused by spike RBD vaccination.[6]

[6] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9244107/

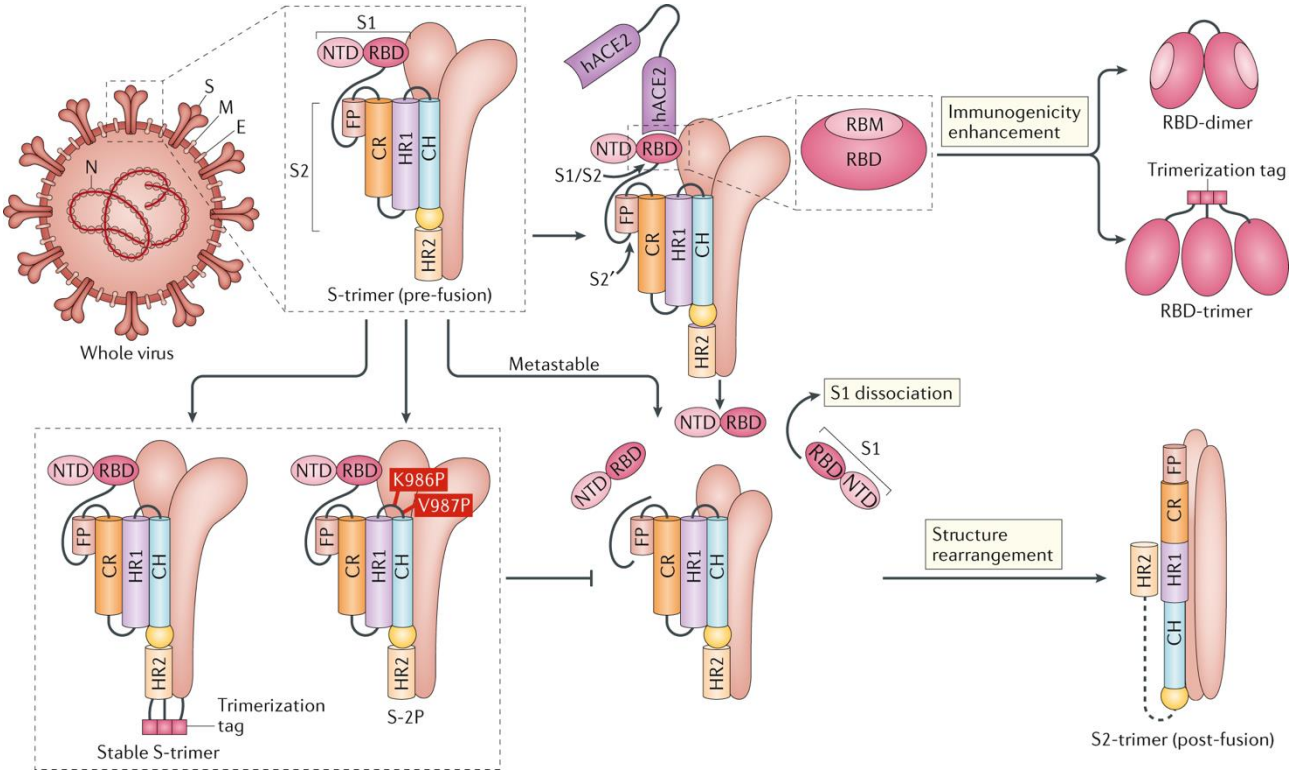


DIFFERENCE BETWEEN NATURAL AND VACCINE SPIKE PROTEIN

As mentioned above, the S protein of SARS-CoV-2 virus functions as a trimer and consists of three identical molecules, which are encoded by the same gene. The S1 subunit can be in two conformations: open and closed, and consequently the RBD domain can be in "up" or "down" conformations. It has been shown that the RBD domain of the S protein of SARS-CoV-2 virus is mainly in the "down" conformation and that the closed conformation form of the protein is weakly immunogenic.

In mRNA vaccines ("Pfizer" and "Moderna" vaccines), mutation of S protein residues 986 and 987 to proline produces a stabilized S antigen in the prefusion conformation (S-2P).
Specifically, after expression of the BNT162b2 coding sequence in cells, it was seen that a portion of the molecules stably presented one RBD in the "up" conformation (accessible for receptor binding), and two RBDs "down" (closed conformation).[7]
The rigidity of this conformation (largely closed and thus favoring the formation of low-affinity antibodies to the RBD) and the sequence specificity of the vaccine antigen (obtained by chemical synthesis from the unique sequence of the Wuhan-1 strain) result in the formation of antibodies targeted against this vaccine antigen, which precisely because of their high specificity may be responsible for the selection of vaccine-resistant viral variants[8] and an increased risk of a phenomenon of disease potentiation, which has already been shown for other viruses including SARS-Cov-1, known as ADE (antibody-dependent enhancement.[9]

[7] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8311925/
[8] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8805732/
[9] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8814804/



https://www.nature.com/articles/s41577-020-00480-0/

THE CONCEPT OF QUASISPECIES AND DISEASE ENHANCEMENT

- QUASISPECIES

A major factor in the evolutionary success of RNA viruses is the use of error-prone replication, which generally lacks proofreading mechanisms. Average mutation rates are about 10^{-4}–10^{-5} errors per copied nucleotide or, depending on average genomic size, about one mutation per copied genome. Consequently, a viral population does not exist as a collection of genetically identical sequences, but rather as a collection of closely related mutants defined as quasispecies. The quasispecies theory has important repercussions for RNA virus evolution and adaptability. As a large population of randomly generated mutants, a quasispecies viral population will have a wide range of phenotypic variation. Genetic recombination provides additional sources of genetic variation. Having a large amount of preexisting variation would therefore lead to rapid adaptation in response to changes in the environment, and viral quasispecies may already contain resistant mutations even before selective pressure is applied. The high mutation rates found in an RNA quasispecies also increase the likelihood of the generation of immune evasion mutations. These evasion mutations would then be selected in the presence of environmental pressures (e.g., antiviral drugs, vaccine or monoclonal antibodies).[10]

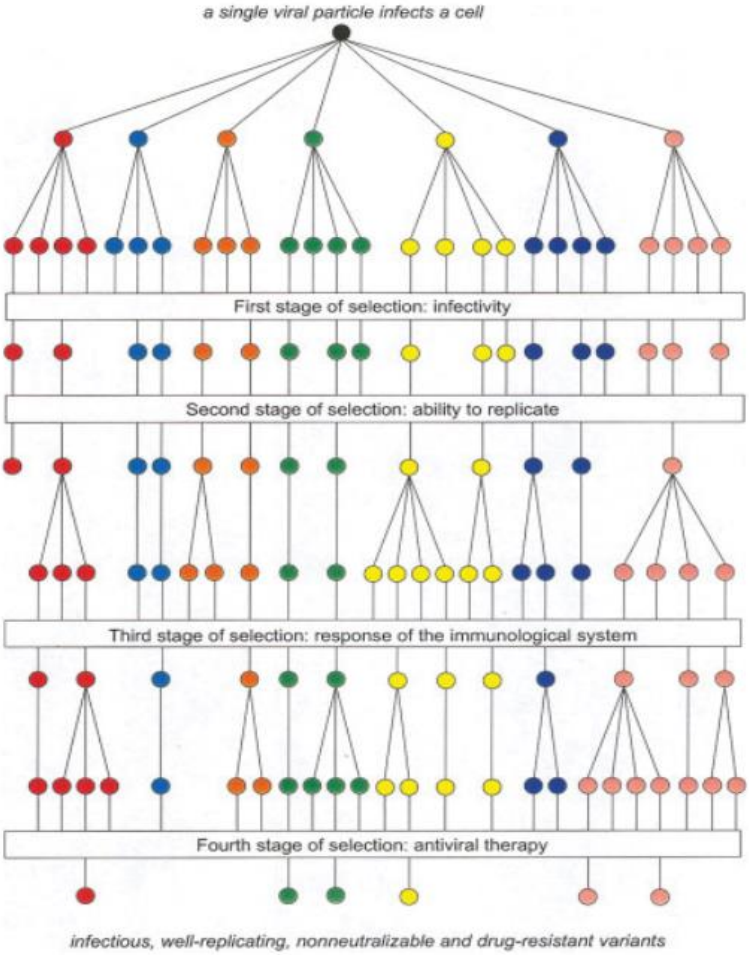
In the diagram below, the mechanism of resistance of RNA viruses to antiviral drugs and vaccines is depicted schematically: during the first phase, a single viral particle (wild-type virus represented by a black dot) infects the cell and begins to replicate. As a result, a large pool of highly diverse viral particles

[10] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4488735/

(represented by colored dots), termed quasi-species, is generated. Virtually each virus differs from the other as well as from the wild-type form. When the viral particles leave the cell, they are subjected to selection by the host organism. First of all, they must be infectious to enter a new cell (first stage of selection). Those viruses that have been able to infect new cells must replicate their genomes and produce progeny; in the other case, the progeny is eliminated (second stage of selection). After a few days, replicating viruses undergo selection by the host immune system. As a result, mutants that evade the immune response are generated in the third stage of selection.

Finally, the infected patient undergoes antiviral therapy, and only drug-resistant mutants are allowed to replicate and spread further (fourth stage of selection). In this way, four-stage selection generates infectious, replicating, nonneutralizing, and drug-resistant viral variants.[11]

It should be noted that in the course of natural infection, the variants that are selected during the third stage of selection depend on the immune response: if the immune system is efficient, progressively asymptomatic variants are selected, leading to tolerance of viral replication in the organism and promoting herd immunity,[12] conversely, if the immune system is compromised, the virus replicates for longer times and even more aggressive variants can be selected.[13]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7168509/pdf/MED-23-488.pdf

[11] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7168509/pdf/MED-23-488.pdf
[12] https://academic.oup.com/cid/article/75/1/e545/6563799
[13] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8494465/

- ANTIBODY-DEPENDENT DISEASE ENHANCEMENT

In the fight against viruses, the immune system has two main weapons, cytotoxic T lymphocytes and neutralizing antibodies, both of which play a key role in controlling viral infections, especially in the case of respiratory viruses. However, virus-specific antibodies can also promote pathology, a phenomenon called antibody-dependent enhancement (ADE). ADE is generally due to virus-specific antibodies that facilitate virus entry into host cells and, in some cases, enhance virus replication in monocytes, dendritic cells, and macrophages through antibody binding to Fc receptors, or through alternative mechanisms involving the complement component C1q.
ADE has been observed in two typical situations: (a) reinfection with a viral variant after primary infection with a different strain or a cross-reactive virus, and (b) as a result of viral infection in vaccinated persons. Several pieces of evidence may argue in favor of an ADE problem for SARSCoV-2: (a) ADE has been reported for animal coronaviruses such as feline infectious peritonitis virus and for SARS-CoV-1. In preclinical studies, previously vaccinated animals went into premature death after coronavirus challenge; (b) ADE epitopes have been found in human coronaviruses related to SARS-CoV-2, namely SARS-CoV-1 and MERS -CoV. The case of SARS-CoV-1 is particularly interesting because its protein spike shows a linear ADE epitope, 597-LYQDVNC-603 that is completely conserved in the protein sequence of the SARS- CoV-2 spike protein used for COVID-19 mRNA vaccines.[14]
ADE is most likely to occur when a person is vaccinated with a virus or genetic construct expressing an S-protein with a predominantly open form of RBD is getting infected with a virus with a predominantly closed conformation of this protein.[15]

Types of ADEs

ADE
Antibody-dependent enhancement (ADE) can be mediated by antibody Fc receptor-associated internalization of a virus, thus resulting in more extensive viral replication and cytokine release in the presence of virus-specific antibodies.

ERD
Enhanced respiratory disease (ERD) manifests with more severe clinical symptoms after infection with respiratory viruses, such as respiratory syncytial virus and influenza virus, due to previous immune responses. ERD usually presents with a peribronchiolar monocytic infiltrate with an excess of eosinophils. ERD can occur during infection with homotypic or heterotypic serotype virus infection after vaccination, natural infection, or transfer of maternal passive immunity.

VADE
Vaccine-associated disease enhancement (VADE) partially overlaps with ADE and ERD. In contrast to ERD, VADE involves only the vaccine-associated reaction and, more importantly, is not limited to respiratory disease. For example, heterotypic serotype Dengue virus infection can cause more severe Dengue hemorrhagic fever in vaccinated individuals. This phenomenon is related to VADE but does not include ERD. VADE can be attributed to antibody-dependent and type 2 T helper cell-dependent mechanisms.[16]

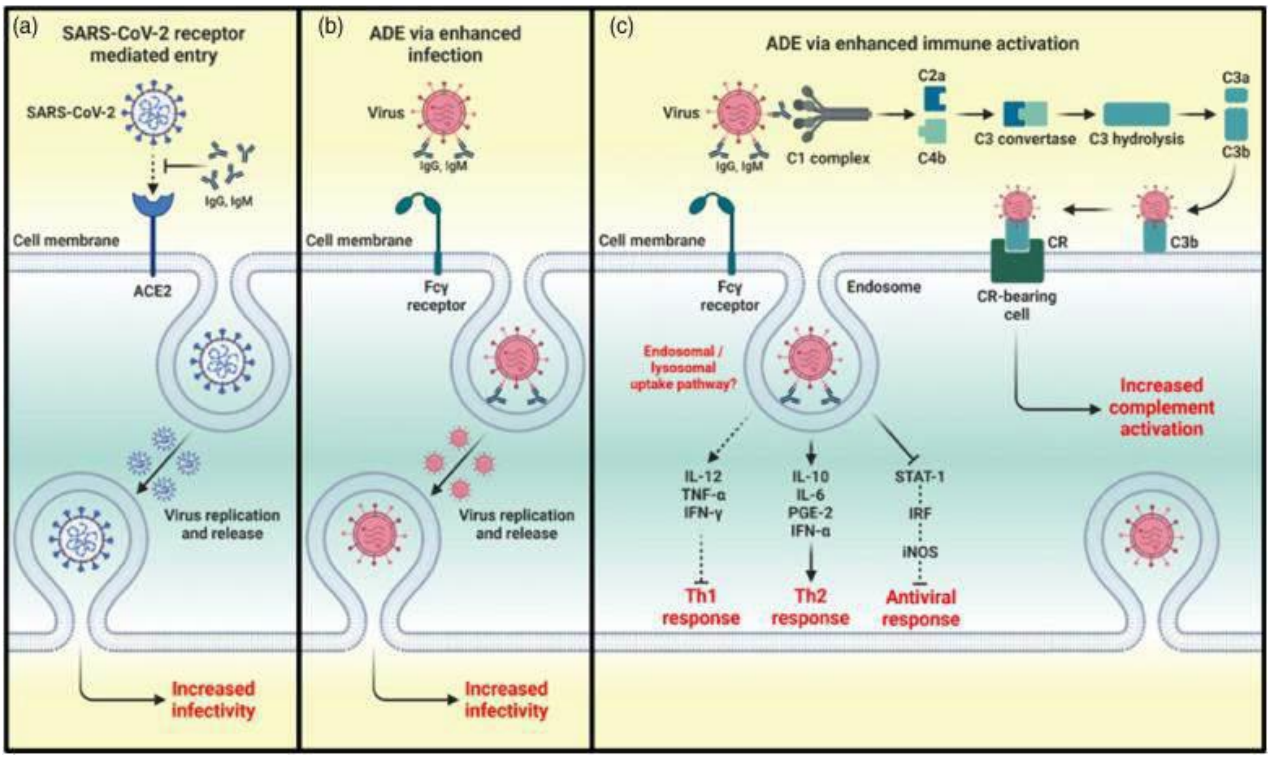
[14] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9412366/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9230616/
[15] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7569100/
[16] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9548747/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8570879/

ADE via enhanced infection

As mentioned above, FcRs are mainly expressed by immune cells and are receptors directed to Fc portion of an antibody. In ADE mediated by enhanced infection, non-neutralizing or sub-neutralizing antibodies bind to the viral surface, forming an immune complex that is internalized by Fc receptor-carrying cells, including monocytes/macrophages and dendritic cells, and induce activation of the signal cascade for FcγR-mediated phagocytosis, which consequently results in increased viral load and disease severity.

Complement-mediated ADE

Complement-mediated ADE (C-ADE) occurs when the combination of virus and antibody forms an immune complex following complement activation, binds to complement to form a complex (virus-antibody-complement), and then enters the cell promoting infection.
Not only the cellular entry of immune complexes, but also extracellular complexes (virus-antibody-complement) can activate complement pathways through deposition in airway tissue, which further induces recruitment and activation of neutrophils, monocytes, and eosinophils and stimulates the production of a number of pro-inflammatory cytokines. In addition, uncontrolled complement activation always contributes to disseminated intravascular coagulation (DIC), inflammation, immune system cell death, immune paralysis, and eventually leads to multi-organ failure and death.[17]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8512237/

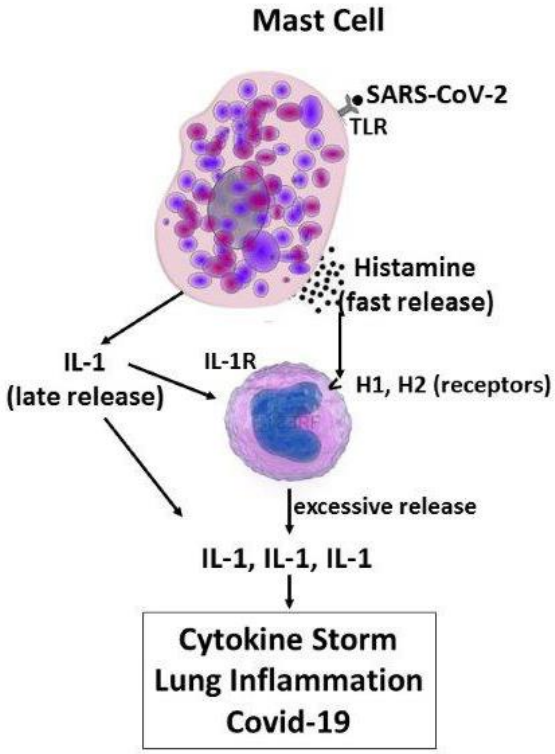
[17] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9647202/

ADEs and mast cells

Mast cells are tissue-resident cells containing mediators that can regulate both innate and adaptive immune responses and are classically known for their IgE response cross-linked the FcεR1 receptor, which is important in protective immunity against helminth infection and pathologically associated with allergic disease. However, mast cells are also important tissue sentinel cells for initiating the inflammatory response to pathogens.
Mast cells have two distinct phases of activation: immediate degranulation, resulting in the release of pre-synthesized mediators (histamine, TNF-alpha, tryptase and chymase, amines), and delayed secretion of *de novo* synthesized secondary mediators.
Delayed secretion of *de novo* secondary effector molecules can be further divided into two classes:

- prostaglandins and eicosanoids released within minutes of activation,
- cytokines, chemokines and growth factors that are released within hours of activation.

Together, these mast cell secretions can increase epithelial and endothelial cell permeability and activation state, which together with chemotactic molecules cause increased of inflammatory cells recruitment into infected tissues.[18]



https://www.researchgate.net/publication/344315820_Mast_cells_activated_by_SARS-CoV-2_release_histamine_which_increases_IL-1_levels_causing_cytokine_storm_and_inflammatory_reaction_in_COVID-19

Antibody-mediated mast cell activation can occur following various infections and vaccines, and has been most carefully studied as an immunopathologic mechanism for MIS-C (multisystem inflammatory

[18] https://pubmed.ncbi.nlm.nih.gov/32945158/

syndrome in children) and MIS-A (multisystem inflammatory syndrome in adults) as a complication of COVID-19 and SARS-Cov-2 vaccines.
In particular, MIS-C appears to be a clinical syndrome that shares aspects with other inflammatory conditions, in which large amounts of cytokines cause dysfunction of several organs, including Kawasaki disease, sepsis, macrophage activation syndrome, and secondary HLH. Its action on the vascular bed is very important, as it causes hypotension and leakage of fluid and immune system cells into the lung, heart, and other organs.
Of note is cardiac involvement with myocardial dysfunction, pericarditis, valvular dysfunction, or coronary artery abnormalities. These increased histamine levels are predicted to impede blood flow through cardiac capillaries due to constricted pericytes with increased risk for cardiac pathology due to cell death by anoxia and coronary artery aneurysms due to increased blood pressure.[19]

TOXIC SEQUENCES OF THE SPIKE

Homology between protein sequences of the SARS-CoV-2 spike and neurotoxins of animal origin (from the genera *Ophiophagus* (cobra) and *Bungarus*, as well as the neurotoxin-like regions of three rabies virus strains) known to be high-affinity antagonistic acetylcholine competitors for α7 nAChR (nicotinic receptors) has recently been reported.
The insertion PRRA together with seven sequentially preceding residues and succeeding R685 (conserved among β-CoVs) form a motif, Y_{674}QTQTNSPRRAR$_{685}$, homologous to those of neurotoxins from *Ophiophagus* (cobra) and *Bungarus genera*, as well as the neurotoxin-like regions from three RABV.
Peptides related to this sequence were found in plasma and stool samples from COVID-19 patients, and their presence was related to extrapulmonary symptoms of COVID patients and long Covid cases. In particular, the presence of conotoxin peptides could explain the occurrence of many symptoms (such as hyposmia, hypogeusia, and the typical signs of Guillain-Barre syndrome) observed in some COVID-19 patients. Their presence may alter the normal functioning of ion channels, nicotinic acetylcholine receptors and acetylcholine levels.[20]
In addition, study of the effects of viral spike and toxic peptides on in vitro cultures of pluripotent nerve stem cells has demonstrated altered expression of genes critical for nervous system development and decreased spontaneous electrical activity.[21]
Further bioinformatic analysis showed that the same segment has close similarity to the SAg (Superantigen) motif F164 to V174 of the HIV-1 glycoprotein gp120, and there is sequence homology between the T678 and Q690 fragment of the SARS-Cov2 spike and the SEB superantigen peptide T_{150}NKKKATVQELD$_{161}$. This dodecapeptide sequence shows strong conservation in a wide range of staphylococcal and streptococcal SAg.
SEB (staphylococcal enterotoxins B) allows widespread activation and proliferation of T cells, resulting in massive production of proinflammatory cytokines including IFNγ, TNFα, and IL-2 from T cells and IL-1 and TNFα from antigen-presenting cells. This cytokine storm leads to multiorgan damage such as that seen in MIS-C.

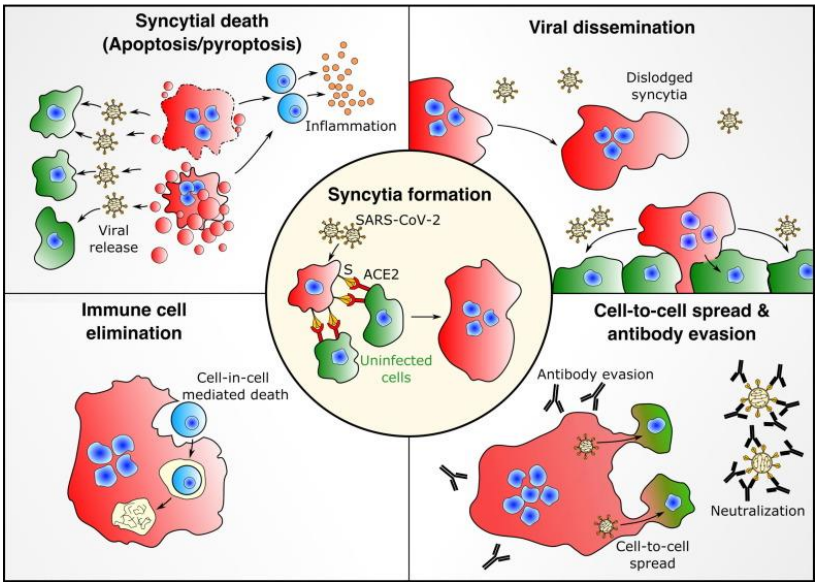
[19] https://scientonline.org/open-access/etiology-scenarios-for-multisystem-inflammatory-syndrome-in-children-and-adults-associated-with-sars-cov-2.pdf
https://www.pediatricsresearchjournal.com/articles/kawasaki-disease-multisystem-inflammatory-syndrome-in-children-antibody-induced-mast-cell-activation-hypothesis.html
https://www.frontiersin.org/articles/10.3389/fimmu.2021.640093/full
[20] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8772524/
[21] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9068247/

The results suggest that the hyperinflammatory syndrome originates from the superantigenic S-glycoprotein activity of SARS-CoV-2, and raise the possibility that the hyperinflammation observed in severe cases of COVID-19 in adults may also be activated by the SAg-like action of the S protein.[22]

SPIKE AND SYNCYTIAL FORMATION

Syncytia are the product of fusion between two or more cells and have been found in COVID patients with severe and extensive lung damage as infected multinucleated syncytial pneumocytes.
These syncytials have been attributed to the ability of the spike to fuse the membrane of the host cell with the membrane of an adjacent cell if the latter cell also has a receptor for the spike.
The SARS-CoV-2 spike can fuse cells even if the virus is not infectious, or if the spike is incorporated into extracellular vesicles (exosomes) released by infected cells.

This mechanism is known as fusion from outside, as the viral particle or a vesicle provides a bridge between membranes. Because the syncytium produced by this mechanism is not infected with SARS-CoV-2 in this case, its origin may be difficult to trace. This also means that extracellular vesicles produced in patients with COVID-19 may be able to form syncytia and cause thrombosis either locally, by fusing infected cells, or remotely via exosomes that carry the spike even in tissues that are not infected with the virus.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8485708/

In the case of SARS-Cov-2, the spike has been detected in the brains of deceased patients with COVID-19, and given how efficiently SARS-CoV2 spike fuses cells and how intricate the neuronal networks are, the possibility that the spike could disrupt them by fusing some of their components together is not negligible, as has also been shown in a recent study in brain organoids.
It was found that the four-amino acid insert (PRRAR) of furin before the S1/S2 cleavage site present only in the SARS-CoV-2 spike is responsible for the ability to fuse cells, including cardiomyocytes.[23]

[22] https://www.pnas.org/doi/10.1073/pnas.2010722117
[23] https://pubmed.ncbi.nlm.nih.gov/34613786/
https://www.nature.com/articles/s41418-021-00782-3

Since the Covid vaccines used to date retain in part the fusogenic properties of the viral spike, and exosomes carrying the vaccine spike are found in circulation, it is conceivable that various types of vaccine damage may also be triggered by this mechanism.[24]

PRION PROPERTIES

It is widely recognized that misfolding of human prion and prion-like proteins plays a causative role in a large and growing number of neurodegenerative diseases. It has been shown by several studies that the SARS-CoV-2 spike protein contains extended amino acid sequences previously established as characteristic of a prion-like protein. This suggests that vaccine-induced spike protein production involves prion-like protein with its pathological consequences such as neuroinflammation, neurodegenerative diseases, and coagulation disorders within the vascular system.[25]

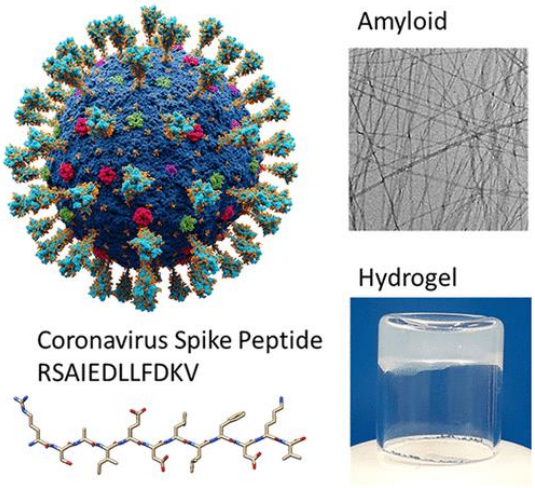
A study that evaluated the amyloidogenic potential of the spike protein used both theoretical and experimental methods to test whether the SARS-CoV-2 spike protein could cause the appearance of amyloid-like fibrils after the protein underwent proteolysis. Theoretical predictions identified seven potentially amyloidogenic sequences within the spike protein. In laboratory experiments in which the protein was incubated with the neutrophil protease elastase, amyloid-like fibrils appeared during 24 hours of coincubation. One specific segment, spike 194-213 (FKNIDGYFKI) was very abundant after six hours and overlapped almost completely with the most amyloidogenic sequence identified theoretically. Neutrophils responding to immune activation release neutrophil elastase into the medium, where it would have access to the spike protein and be able to break it down into the amyloidogenic segments.[26]

Lewy bodies are protein aggregates that accumulate in the brain in association with Parkinson's disease and other neurodegenerative diseases. A study published in 2022 found experimentally that the spike protein interacts with amyloidogenic proteins, particularly α-synuclein, a causative factor in Parkinson's disease (PD), and induces Lewy body-like pathology in a cell line.[27]

A preprint paper co-authored by Prof. Montagnier describes 26 cases of patients with severe CJD symptoms shortly after a COVID-19 vaccine. Twenty-three of 26 cases developed symptoms within 15 days after the second injection of an mRNA vaccine. The other three cases were associated with the AstraZeneca adenoviral vector vaccine and symptoms appeared within the first month. Of the 26, 20 were dead at the time of writing, and the remaining 6 were in critical condition. The mean time to death was less than five months after injection.[28]

In addition, blood clotting associated with extracellular amyloid fibrillar aggregates in the bloodstream has been reported in patients with COVID-19. Altered hypercoagulation/impaired fibrinolysis has been demonstrated in blood plasma from healthy donors experimentally supplemented with protein S.[29]

[24] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8664391/
[25] https://www.authorea.com/users/455597/articles/582067-sars-cov-2-spike-protein-in-the-pathogenesis-of-prion-like-diseases
[26] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9136918/
[27] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8949667/
[28] https://zenodo.org/record/7304759#.Y-4gci1Q10s
[29] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8380922/



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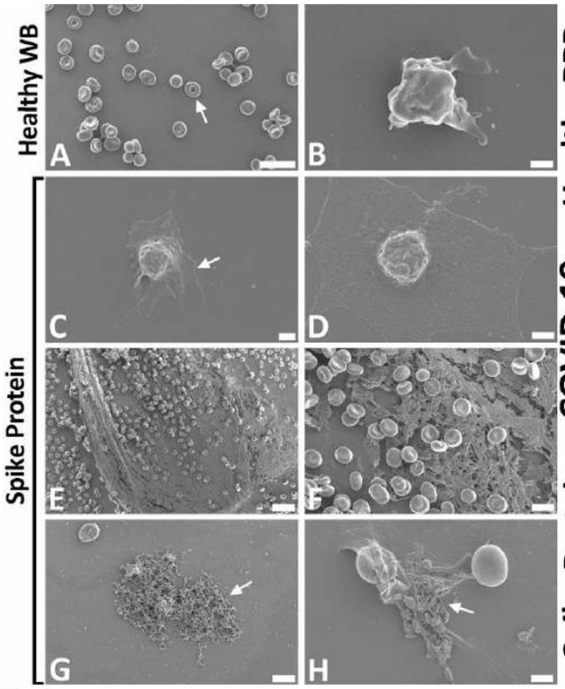


Fig. 1

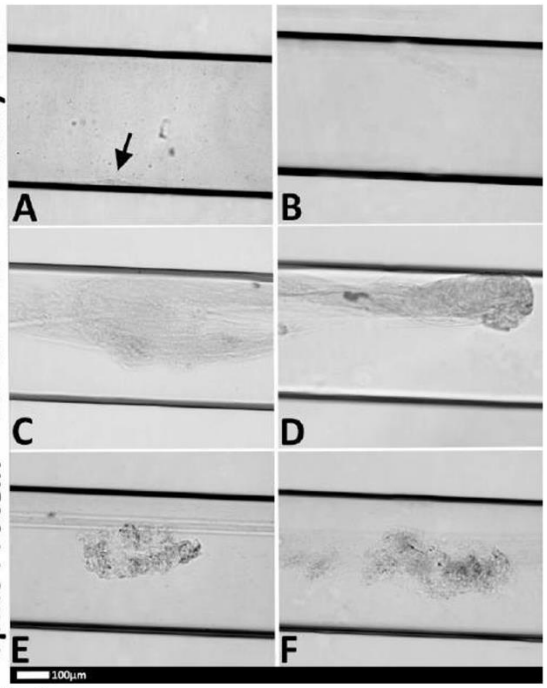


Fig. 2

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8380922/

Fig. 1

Whole blood sample of healthy volunteers, before and after exposure to spike protein

(**A–H**) Representative scanning electron micrographs of healthy control WB, with and without spike protein. (A,B) Healthy WB smears, with arrow indicating normal erythrocyte ultrastructure. (C–H) Healthy WB exposed to spike protein (1 ng.ml^{-1}final concentration), with (C,D) indicating the activated platelets (arrow), (E,F) showing the

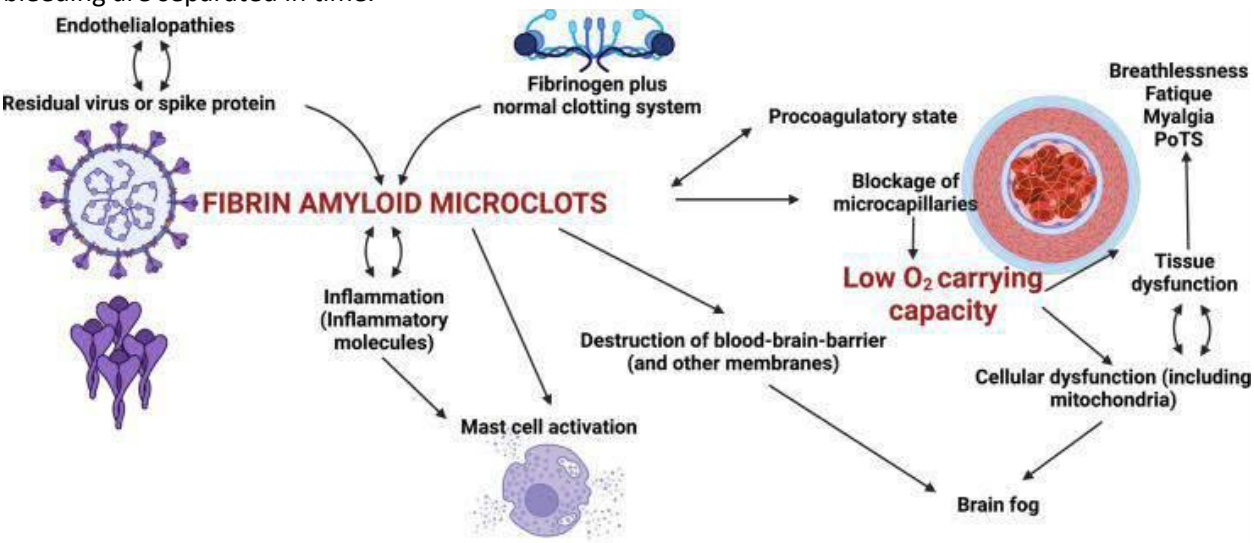
spontaneously formed fibrin network and (G,H) the anomalous deposits that is amyloid in nature (arrows) (scale bars: (E) 20 µm; (A) 10 µm; (F,G) 5 µm; (H) 2 µm; (C) 1 µm; (B,D) 500 nm).

Fig. 2

Representative micrographs of PPP clots in the microfluidic chambers (black horizontal lines are the contours of the chambers) that were coated with thrombin

(**A**) Healthy PPP clot, with small clot formation (arrow), with (**B**) no clot formed in the healthy PPP sample. (**C,D**) examples of clots from COVID-19 PPP samples and (**E**,**F**) healthy PPP clot with spike protein. Black arrow = small clot formed in control sample; red arrows large clots in COVID-19 sample.

Fibrinogen in the blood can coagulate into an abnormal "amyloid" form of fibrin that (like other amyloids and β-rich prions) is relatively resistant to proteolysis (fibrinolysis). The result, as occurs in the platelet-poor plasma (PPP) of individuals with long-COVID, are extensive fibrin amyloid microclots that can persist, trap other proteins, and lead to the production of various autoantibodies.
Another feature of COVID-19 is extremely high levels of platelet activation due to dysregulation of P-selectin (P-selectin is an inflammatory biomarker of coagulation and is known to modulate interactions between blood cells and endothelial cells), and hyperferritinemia.
This leads to an apparent paradox, where both coagulation and bleeding can be observed as part of the pathology, and it is believed that the resolution of the paradox is that these stages of coagulation and bleeding are separated in time.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8883497/

The difference between vaccine mRNA and the viral genome regarding the ability to form spikes in prion form is related to the "codon optimization" step of vaccine mRNA. This genetic modification consists of the replacement of codons coding for amino acids used by the virus with others that are more efficient in protein assembly. In the case of commercial mRNA vaccines, the more efficient codons contain on average more guanines than other codons.
Guanine nucleotides, when enriched in the nucleotide sequence, are sometimes able to configure themselves into a tertiary structure called a "G quadruplex" (G4), and it is known that human prion protein mRNA contains multiple G4-forming motifs, which can play a critical role in causing the prion protein to assume a pathological conformation.[30]

[30] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4132711/

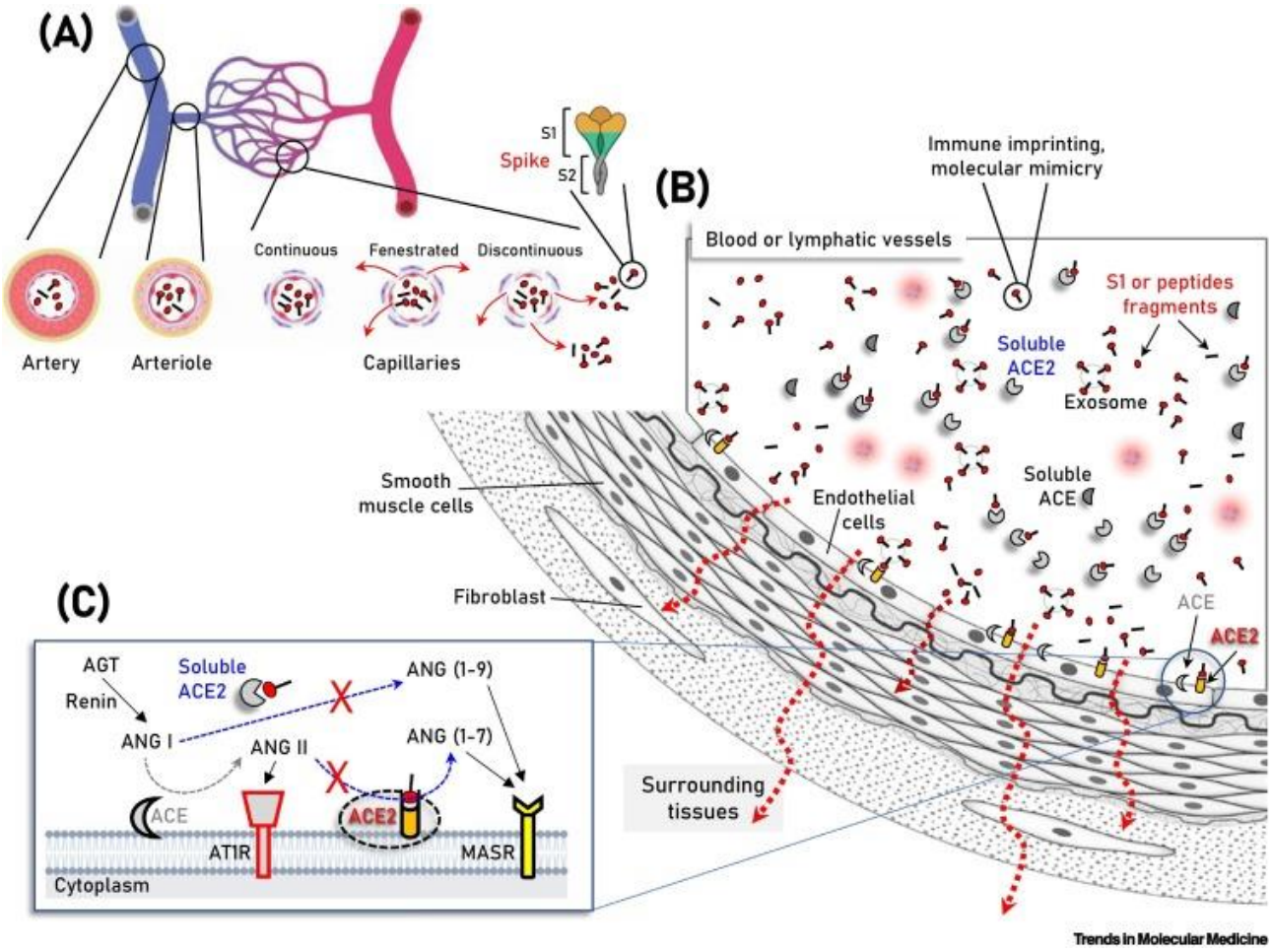
The original nucleotide sequence in the viral version of the protein spike mRNA has the potential to form four G4 motifs, while the Pfizer version has the potential to produce nine, and the Moderna version can form 19,[31] confirming the increased risk of vaccine spike formation in the pathological prion form.

ENDOTHELIAL DAMAGE

It has been suggested by several studies that endothelial damage is a central part of SARS-CoV-2 pathology that can be induced by protein S alone. In fact, intravenous (iv) injection of the S1 subunit into mice resulted in its localization in the endothelium of the brain microvessels of mice showing colocalization with ACE2, caspase-3, IL-6, tumor necrosis factor α (TNF-α) and C5b-9. In addition, the S1 subunit (or recombinant S1 RBD) was able to impair endothelial function through underregulation of ACE2 and induce degradation of junctional proteins that maintain the integrity of the endothelial barrier in a mouse model of brain microvascular endothelial cells or cerebral arteries. Similarly, S1 subunit decreased microvascular transendothelial resistance and barrier function in cultured human pulmonary cells and disrupted the function of human cardiac pericytes, triggered increased production of proapoptotic factors in pericytes, and caused endothelial cell death.

The potential interaction at a whole-organism level of the native-like S protein and/or subunits/peptide fragments with soluble or cell-membrane-attached ACE2 can promote ACE2 internalization and degradation. In support of this, soluble ACE2 has been shown to induce SARS-CoV-2 receptor-mediated endocytosis through interaction with RAS-related proteins. Prolonged loss or reduced activity of ACE2 can result in extensive destabilization of the RAS that may then trigger vasoconstriction, enhanced inflammation, and/or thrombosis due to unopposed ACE and angiotensin-2 (ANG II)-mediated effects. Indeed, decreased expression and/or activity of ACE2 contributes, among other things, to the development of ANG II-mediated hypertension in mice, indicating vascular dysfunction. These effects, especially in capillary beds, and the prolonged presence of the antigen in the circulation, together with the excessive systemic immune response to the antigen, may then trigger prolonged inflammation that can damage the endothelium, disrupting its antithrombogenic properties in multiple vascular beds.[32]

[31] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9012513/
https://osf.io/bcsa6/
[32] https://www.mdpi.com/2227-9059/11/2/451



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Schematic of the vasculature components showing vaccination-produced S protein/subunits/peptide fragments in the circulation, as well as soluble or endothelial cell membrane-attached angiotensin-converting enzyme 2 (ACE2). (A,B) Parallel to immune system activation, circulating S protein/subunits/peptide fragments (B) binding to ACE2 may occur not only to ACE2-expressing endothelial cells, but also in multiple cell types of the vasculature and surrounding tissues due to antigen diffusion (e.g., in fenestrated or discontinuous capillary beds) (A, red arrows). These series of molecular events are unlikely for any severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-related antigen in the absence of severe coronavirus disease 2019 (COVID-19), where SARS-CoV-2 is contained in the respiratory system. In (C) the two counteracting pathways of the renin–angiotensin system (RAS), namely the 'conventional' arm, that involves ACE which generates angiotensin II (ANG II) from angiotensin I (ANG I), and the ACE2 arm which hydrolyzes ANG II to generate angiotensin (1–7) [ANG (1–7)] or ANG I to generate angiotensin (1–9) [ANG (1–9)] are depicted. ANG II binding and activation of the ANG II type 1 receptor (AT1R) promotes inflammation, fibrotic remodeling, and vasoconstriction, whereas the ANG (1–7) and ANG (1–9) peptides binding to MAS receptor (MASR) activate antifibrotic, anti-inflammatory pathways and vasodilation. Additional modules of the RAS (i.e., renin and angiotensinogen, AGT) are also shown. Abbreviation: AT1R, angiotensin II type 1 receptor.

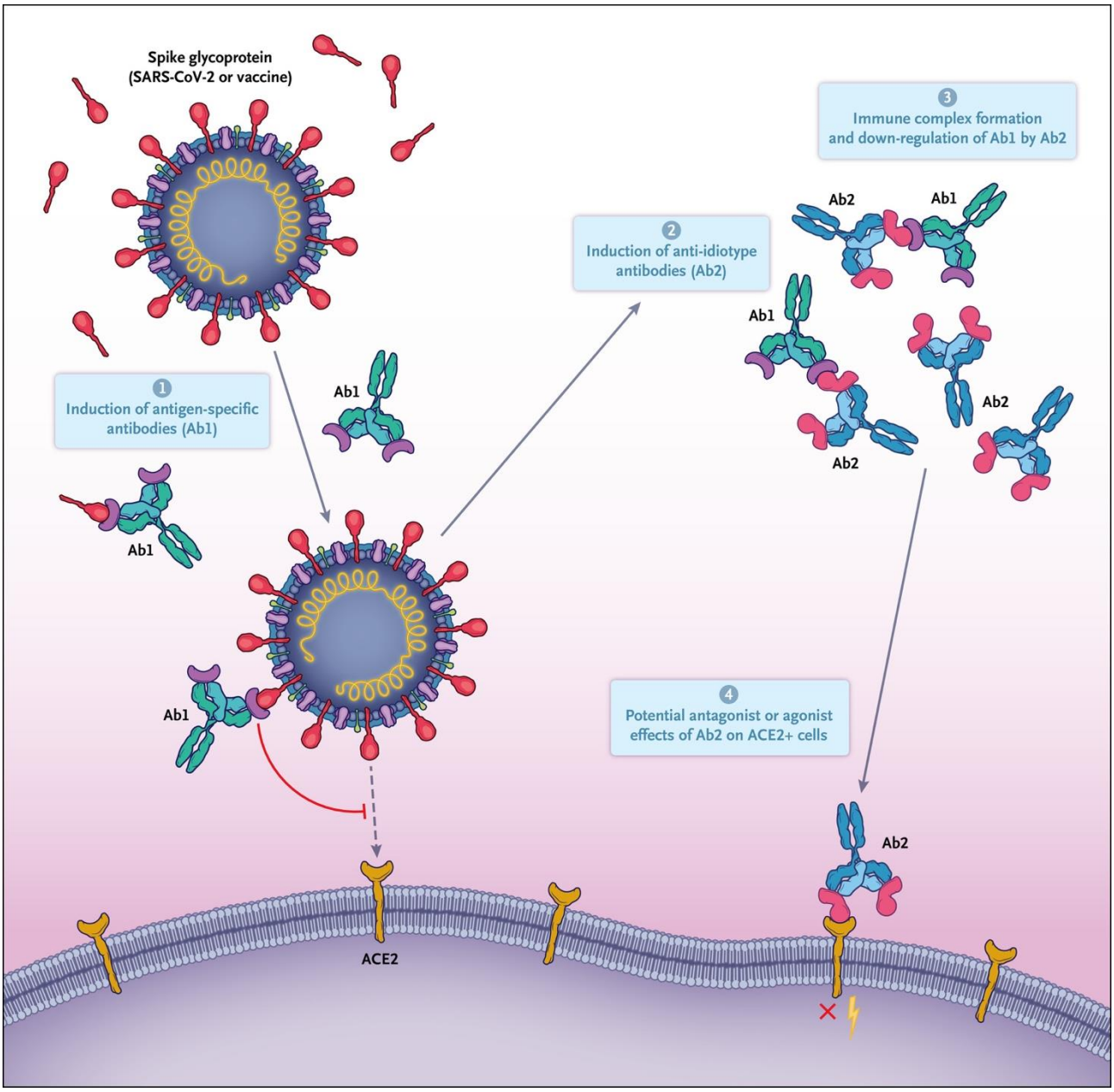
After vaccination, a cell may present the produced S protein (or its peptide subunits/fragments) to mobilize immune responses or be destroyed by the immune system (e.g., by cytotoxic T cells). Consequently, the debris produced, or even the direct secretion (including shedding) of the antigen by the transfected cells, can release large amounts of the S protein or its peptide subunits/fragments into the circulation. The anti-SARS-CoV-2 vaccine mRNA-containing LNPs are injected into the deltoid muscle and exert an effect in the muscle tissue itself, the lymphatic system, and the spleen, but can also localize

in the liver and other tissues from where the S protein or its subunits/peptide fragments may enter the circulation and distribute throughout the body.
In line with a plausible systemic distribution of the antigen, it was found that the S protein circulates in the plasma of the BNT162b2 or mRNA-1273 vaccine recipients as early as day 1 after the first vaccine injection. Reportedly, antigen clearance is correlated with the production of antigen-specific immunoglobulins or may remain in the circulation (e.g., in exosomes) for longer periods, providing one reasonable explanation (among others) for the robust and durable systemic immune responses found in vaccinated recipients. Therefore, there is likely to be an extensive range of expected interactions between free-floating S protein/subunits/peptide fragments and ACE2 circulating in the blood (or lymph), or ACE2 expressed in cells from various tissues/organs.[33]

MOLECULAR MIMICRY

Spike protein has some common motifs with human proteins, including a five-amino acid stretch (TQLPP) with antigenic properties that are homologous with a sequence found in thrombopoietin, and the ELDKY motif that is shared with tropomyosin and with Protein Kinase cGMP-dependent type 1 (PRKG1), a kinase involved in platelet activation and calcium regulation.
Molecular mimicry is one of the mechanisms hypothesized to explain the development of autoimmune disease. An important concern is whether mRNA vaccination for Spike protein production could result in a breakdown in tolerance and the development of autoimmune disease due to molecular mimicry. The risk increases with frequent and close administrations of the vaccine, which challenge the immunogenic versus tolerogenic state of the immune system.[34]
In this condition, proinflammatory cytokines can alter the control of immunoregulatory circuits so that autoreactive T cells can become effective and trigger autoimmunity. In addition, the "homologies" between the Spike protein and human proteins are much greater than for other viruses and bacteria, increasing the risk of developing autoimmune diseases.
The issue of interactions between the immune system and ACE2 (or other virus receptors) is further complicated when considering that the immune system is complex and dynamic.
For example, the formation of anti-idiotype antibodies and lymphocytes is one possible explanation for the persistence of typical COVID-19 symptoms even after the virus has been eliminated from the body. Indeed, anti-idiotype antibodies (Ab2 in the Figure below), which reflect the Spike epitope, can bind to ACE2 or similar structures and cause the pathophysiological reaction of long Covid.
This phenomenon can occur with SARS-CoV-2 infection as well as with anti-COVID-19 vaccines, explaining at least in part the persistence of adverse reactions in some individuals. It has also been suggested that anti-idiotype antibodies might bind to neuropilin-1, which is recognized by the SARS-CoV-2 virus Spike, and this might explain some neurological adverse effects such as the peripheral neuropathy that arises after vaccination with BNT162b2. [33, 35]

[33] https://www.mdpi.com/2227-9059/11/2/451
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9021367/
[34] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9318917/
[35] https://pubmed.ncbi.nlm.nih.gov/34818473/



https://pubmed.ncbi.nlm.nih.gov/34818473/

To summarize, the mechanisms by which the free Spike protein can act in living systems are listed in the Table below.[33]

Molecular Mechanisms	Pathogenic Mechanisms	Possible Clinical Effects
Spike-ACE2	Platelet hyperreactivity and aggregation	Thrombosis
Spike-ACE2	Human endothelial cell activation and pro-inflammatory phenotype	Inflammation, thrombosis
Spike-ACE2	Inhibition of hematopoietic stem cells differentiation	Immunosuppression
Spike (S1)-ACE2	Intratracheal S1 subunit of Spike protein in hACE2 transgenic mice that overexpress human ACE2	Lung vascular permeability and lung injury
Spike-ACE2	Mast cell activation	Lung inflammation and injury
Spike-ACE2	Oxidative stress in pericytes, activation of nuclear factor-kappa-B signaling pathways	Encephalitis
Spike-ACE2	Down-regulation of endothelial ACE2 and e-NOS, mitochondrial damage	Interstitial pneumonia
Spike-ACE2	Decrease of type I interferons in lung primary cells	Severity of pneumonia
Spike (S1)-ACE2	S1 subunit co-localized with caspase-3, ACE2, IL6, TNFα, and C5b-9 (mice brain endothelia)	Inflammation and neuropathology
Spike (S1)-ACE2	S1 subunit elicits MEK/ERK pathway cell signaling in lung vascular cells.	Pulmonary vascular wall thickening, pulmonary hypertension
Spike-ACE2	Decrease of taste buds of rat circumvallate papillae	Taste disorders
Spike-ACE2	Loss of integrity of the human brain-blood barrier	Pro-inflammatory response on brain
Spike (S1)-ACE2	Loss of integrity of human pulmonary arterial endothelial cells	Pro-inflammatory response on lung
Spike-sACE2-antibodies	Soluble ACE2 internalization and clearance	Hypertensive crisis, inflammation, bradykinin storm
Spike-CD147	Cell signaling in human cardiac pericytes, secretion of cytokines, apoptosis	Cardiac microvascular damage
Spike-CD147	Cell signaling in human platelets	Thrombosis, inflammation
Spike-PAF	Augmentation of in vitro PAF-induced platelet aggregation and stimulation of U-937 (myeloid lineage) PAF production	Inflammatory syndromes, long COVID-19
Molecular mimicry	Cross-reaction of anti-Spike antibodies with pericardium	Pericarditis
Molecular mimicry	Cross-reaction of anti-Spike antibodies with thrombopoietin and with tropomyosin	Thrombocytopenia, myocarditis
Spike-autoantibody	Thyroid inflammation	Subacute thyroiditis
Spike-PF4 interaction	Generation of anti-PF4 antibodies and binding to platelet ACE2	Thrombosis with thrombocytopenia
Anti-PF4 antibodies	Platelet activation and aggregation	Thrombosis with thrombocytopenia
Anti-idiotype	Anti-idiotype (Ab2) would bind to ACE2 and/or to neuropilin-1	COVID-19-like symptoms
Gene expression	Decrease of ACE2 and increase of ACE	Inflammation, myocarditis
Spike-TLR4	The S protein triggers TLRs and induces inflammatory cytokines	Worsening of inflammatory reactions
Immune imprinting	Vaccine immune memory against S protein of the original variant inhibits the response to new epitopes of SARS-CoV-2	Increased susceptibility to COVID-19 variants

https://www.mdpi.com/2227-9059/11/2/451

Molecular, cellular, and immunological mechanisms of pathogenic effects of free Spike protein. A synopsis of the studies reporting the possible clinical effects, and the underlying mechanisms, caused by the expression of the Spike protein either coded by SARS-CoV-2 or the mRNA vaccine. Mechanisms include the molecular interaction of the S protein with membrane-bound or soluble peptides (e.g., ACE2, sACE2, CD147, PAF, PF4, TLRs), the molecular mimicry, the induction of autoantibodies and of anti-idiotype antibodies, altered gene expression, alternative splicing and immune printing (for details, refer to the references). PAF, Platelet Activating Factor; PF4, Platelet Factor 4; TLR, Toll-like receptor.