SARS-CoV-2 Spike Protein in the Pathogenesis of Prion-like Diseases

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Abstract: Human prion protein and prion-like protein misfolding are widely recognized as playing a causal role in a large and growing number of neurodegenerative diseases. Here we summarize the compelling evidence that the spike protein of SARS-CoV-2 contains extended amino acid sequences previously established as characteristic of a prion-like protein. This suggests that vaccine-induced spike protein production is synonymous with production of a prion-like protein, and we trace some of the various pathways through which these proteins should be expected to traverse and distribute throughout the body. We describe some of the highly concerning biological consequences that would be expected to occur with increased frequency as a consequence. Specifically, we describe spike-protein contribution, via its prion-like properties, to neuroinflammation and neurodegenerative diseases; to clotting disorders within the vasculature; to suppressed prion protein regulation in the context of widely prevalent insulin resistance; and other health complications it could be expected to induce. We explain why these prion-like characteristics are more relevant to vaccine-related mRNA-induced spike proteins than natural infection with SARS-CoV-2. We conclude with some potentially ominous public health implications and recommendations for investigations of these possibilities.

Keywords: SARS-CoV-2, spike protein, mRNA vaccines, prion disease, microRNAs, Parkinson's disease, amyloidogenesis, CD16, diabetes.

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1. Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of rare, consistently fatal brain diseases that affect both animals and humans. They are caused by `proteinaceous infectious particles' which can facilitate disease spread in the absence of a classical infection by a living organism. They include the familiar mad cow disease (bovine spongiform encephalopathy), and scrapie in sheep, as well as chronic wasting disease (CWD) in deer. The primary human prion disease is known as Creutzfeldt Jakob disease (CJD), and it is always fatal. Fatal familial insomnia (FFI) is a very rare fatal genetic disease caused by certain mutations in the prion protein. In common nomenclature, the naturally folded form of the prion protein is referred to as PrP^{C}, whereas the misfolded form is called PrP^{SC} (for "scrapie"). Disease propagation occurs through an autocatalytic process whereby external misfolded prion proteins (PrP^{SC}) act as an infectious agent to facilitate misfolding of the same protein expressed in neurons. It is now generally recognized that an intermediate soluble oligomeric form of the protein is the toxic agent, whereas the insoluble plaque may even be protective in that it results in clearing of the soluble oligomers [1].

It is increasingly becoming apparent that there is a generalization of prion diseases that can encompass neurodegenerative diseases such as Alzheimer's, Parkinson's disease, and amyotrophic lateral sclerosis (ALS), which are also associated with misfolded proteins that accumulate in plaques and Lewy bodies. These proteins, which are termed amyloidogenic, have also been labelled as "prion-like," and their spread may also have properties that overlap with the classic stricter definition of the prion protein (PrP) [2,3]. For example, researchers are finding that TAR-DNA binding protein of 43 kDa (TDP-43), a protein that misfolds in association with ALS, forms aggregates that propagate between cells in a prion-like manner [4]. Protein aggregates can be transmitted from one cell to another through at least three distinct mechanisms: tunneling nanotubes, secretion as naked aggregates, or through packaging up into extracellular vesicles such as exosomes.

A remarkable study involved a bacterial plasmid initiator protein, RepA, that builds intracellular amyloid oligomers triggering a lethal cascade in bacteria, similar to the mitochondrial impairment of human cells in neurodegeneration [5]. In this study, the authors worked with a murine neuroblastoma cell line that had been engineered to express wild-type RepA. They were able to demonstrate that *in vitro*-assembled amyloid fibers derived from a mutated form of RepA could infect the neuroblastoma cells and induce the formation of cytotoxic amyloid particles, through propagation of the amyloidicity to the wild-type RepA already present in the cells. Based on their results, these authors stated a "central principle of underlying prion biology:" "No matter the biological origin of a given prion-like protein, it can be transmitted to a phylogenetically unrelated recipient cell, provided that the latter expresses a soluble protein onto which the incoming protein can readily template its amyloid conformation." [5]. They stated that the intercellular exchange of prion-like protein aggregates can be a common phenomenon.

2. The Spike Protein is Prion-like

The COVID-19 mRNA vaccines are based on lipid nanoparticles containing mRNA encoding the SARS-CoV-2 spike glycoprotein. The vaccine has been engineered in several ways to protect the mRNA contents from breakdown, and to assure that cells transfected with it produce large quantities of spike protein at a high production rate over a long time period [6].

A comprehensive study using bioinformatics has identified a large number of viral proteins from diverse species that have prion-like signatures in their genetic sequence. In particular, they identified prion-like domains in viral surface proteins that are involved in receptor binding and fusion with the host cell [7]. These same authors published a later paper analyzing the prion-like potential of the spike protein. They found a prion-like domain in the receptor binding domain (RBD) of the SARS-COV-2 spike protein, which was missing from the original SARS-CoV virus. Asparagine (Q) and glutamine (N)-rich regions are a characteristic feature of many prion proteins. Five amino-acid substitutions in the SARS-CoV-2 variant compared with SARS-CoV formed a hydrophobic Q/N-rich region enabling prionogenesis. They also analyzed some of the SARS-CoV-2 variants, determining that the Delta variant had a higher score for prionogenesis than the original Wuhan strain, whereas Omicron had a substantially lower score [8]. Glutamine-asparagine- rich regions (QNRs) have been found frequently in regulatory molecules and RNA-binding proteins, and are associated with proteins linked to neurodegenerative diseases, including Alzheimer's, Huntington's disease and ALS [9].

A study evaluating the amyloidogenic potential of the spike protein used both theoretical and experimental methods to verify that the SARS-CoV-2 spike protein can cause amyloid-like fibrils to appear after the protein has been subjected to proteolysis. Theoretical predictions identified seven potentially amyloidogenic sequences within the spike protein. In laboratory experiments where the protein was incubated with the protease neutrophil elastase, amyloid-like fibrils appeared during 24 hours of coincubation. A specific segment, spike 194-213 (FKNIDGYFKI) was highly abundant after six hours, and it 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 [10].

Lewy bodies are clumps of protein 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, which is a causative factor in Parkinson's disease (PD), and it induces Lewy-body-like pathology in a cell line [11]. It also induced upregulation of α-synuclein expression. They suggested that this property could be the underlying mechanism accounting for the link between COVID-19 and PD [12].

Prof. Luc Montagnier is a recently deceased Nobel-prize winner for his work on the human immunodeficiency virus (HIV). A preprint paper co-authored by Montagnier describes 26 cases where the patient became severely ill with spontaneous symptoms of CJD shortly after a COVID-19 vaccine. Twenty three out of the 26 cases developed symptoms within 15 days of their second injection of an mRNA vaccine. The other three cases were associated with the AstraZeneca DNA vector vaccine, and symptoms appeared within the first month. Of the 26, 20 had died at the time of writing of the paper, and the remaining 6 were in critical condition. The mean time to death was under five months after the injection [13]. CJD is an extremely rare disease, normally affecting only one in a million people in their lifetime. It also usually takes several years from the time of first onset of symptoms until death. So this is clearly an extraordinarily unusual type of CJD that should raise concern about the safety of these vaccines.

3. A Central Role for the Spleen

As early as 1979, it was recognized that exposure of mice to scrapie prion protein, regardless of whether it was via intraperitoneal, intravenous, or multiple subcutaneous routes, always showed the same pattern of spread of infectivity. Propagation in the spleen consistently showed up well before there was noticeable spread to the spinal cord, with infectivity in the brain requiring the longest incubation period. A conclusion was that spread of the infectivity occurred mainly along nerves rather than via the vasculature or the lymphatic system [14].

Unlike PrP, which is highly expressed in the nervous system but expressed at much lower levels in a plethora of other tissues, the amyloid precursor protein (APP) mRN, is highly expressed in many tissues apart from the nervous system, including muscles, the liver, the immune system (thymus and spleen) and many other organs [15,16].

Only a few studies have been conducted to examine the biodistribution of mRNA from vaccines subsequent to injection. A study published in 2017 tracked distribution of mRNA coding for influenza haemagglutinin proteins, following injection into mouse muscle. They quantified the maximum level of mRNA found in various organs and used this data to infer the migration pathway of the mRNA. As expected, by far the highest concentration remained in the muscle (5,680 ng/mL), but a substantial amount was found in the proximal lymph nodes as well (2,120 ng/mL), with significantly smaller amounts in the distal lymph nodes (177.0 ng/mL). Among the organs, the spleen and liver had by far the highest concentrations (86.9 ng/mL in the spleen and 47.2 ng/mL in the liver). Smaller amounts were found in the plasma (5.47 ng/mL), bone marrow (3.35 ng/mL), ileum (3.54 ng/mL) and testes 2.37 ng/mL, with trace amounts in many other organs including the brain (0.429 ng/mL) [17].

Another study tracked the biodistribution pathway of a rabies mRNA vaccine administered intramuscularly to rats. They found that the mRNA appeared in the draining lymph nodes within one day, and was also found in blood, lungs, spleen and liver [18]. Developers of the technology are pleased to see that the mRNA shows up in the lymph system and the spleen, because T-cell activation and antibody production by B-cells mainly takes place in germinal centers in the lymph nodes and spleen [19].

4. Exosomes and MicroRNAs

Exosomes are membranous secreted nanovesicles 30-150 nm in size, generated and released by all cells, often under conditions of stress. These extracellular vesicles are produced in late endosomes by the inward budding of the endosomal membrane. Their cargo is diverse, and can include nucleic acids, proteins, lipids, and metabolites. They mediate both near and long-distance intercellular communication, through contents that include signaling molecules, nutrients, and toxins. In particular, their lipid membrane can protect internalized RNA molecules from degradation by extracellular ribonucleases. A paper published by Wei *et al.* in 2021 provides an excellent review of the complex mechanisms that control sorting of proteins, RNAs and other molecules into exosomes for export and delivery to other cells [20].

It has been shown experimentally that cells that take up mRNA from the nanoparticles in mRNA vaccines package up some of the mRNA, together with the ionizable cationic lipids, into small lipid particles that are then released into the external medium as exosomes. In fact, these authors found a 1:1 ratio of cationic lipid molecules to nucleotides in the released exosomes [21]. They also demonstrated that cells that took up the exosomes were able to synthesize protein from the mRNA contained in the exosomes. This experiment involved mRNA that codes for human erythropoietin, but a similar result can be expected for the spike-encoding mRNA of the COVID-19 vaccines. In theory, this means that an immune cell in the spleen could ship intact mRNA coding for the spike protein up to the brain along the vagus nerve, and a neuron or microglial cell in the brain could take up the mRNA and begin synthesizing spike protein. In addition, it was shown dramatically in a mouse study published in 2019 that misfolded α-synuclein in the gut can be delivered to the brain via the vagus nerve to cause Parkinson's disease. A vagotomy completely protected the mice from transmission from the gut to the brain [22].

The United States Vaccine Adverse Reporting System (VAERS) is a national vaccine safety surveillance program maintained by the US government where medical practitioners and patients alike can submit cases of adverse reactions they believe were related to any vaccine that they had received [23]. An analysis of data from VAERS involved tabulating the counts in the year 2021 of various adverse events that listed symptoms that could be associated with inflammation in the vagus nerve and/or the major nerves in the head that it connects to. These symptoms included anosmia (loss of smell), tinnitus, deafness, facial palsy, vertigo, migraine headache, dysphonia, dysphagia, nausea, vomiting, dyspnea, syncope and bradycardia. There were altogether over 200,000 cases with these symptoms linked to the COVID-19 vaccines, which represented 97.2% of all the cases for *any vaccine* linked to these symptoms in 2021 [24].

There is also evidence that exosomes play an important role in the propagation of amyloidogenic proteins in the brain. The human prion protein, PrP is found in association with exosomes in both its normal (PrP^{C}) and its misfolded (PrP^{SC}) form. Furthermore, exosomes containing PrP^{SC} are infectious [25]. Exosomes can transport both amyloid β and phosphorylated tau, two proteins that are linked to Alzheimer's disease. The Aβ plaques associated with Alzheimer's are enriched in exosomal proteins, suggesting an original source from exosomes [26]. Techniques that inhibit exosome synthesis have been found to halt tau propagation in a mouse model of tauopathy [27]. Tau protein and Aβ misfolding and coaggregation are found in AD brains, suggesting a universal endocytosis-induced toxicity system operating on these otherwise distinct proteins [28]. Exosomes specifically derived from cells undergoing tau aggregation can seed and corrupt soluble tau in recipient cells [29].

One of the types of molecules often present in exosomes are microRNAs (miRNAs). miRNAs are small single-stranded non-coding RNA molecules containing around 22 nucleotides, that are found across multiple phyla, including animals, plants and viruses. They play an important regulatory role through their ability to silence expression of genes for specific proteins, usually by binding to the 3′ and 5′ (near the 5′ cap) untranslated regions (3′,5′ UTRs) of the mRNA molecule that codes for the protein [30,31].

Both antigen-presenting dendritic cells (DCs) and T cells can secrete and take up exosomal miRNAs, and so it is appropriate to view exosomes as a cell-cell communication mechanism for transferal of these important regulatory RNAs among different cell types, in association with other cargo [32]. Two miRNAs that are important for our discussion here are miR-155 and miR-146a. Both have been found present in exosomes released by immune cells upon exposure to endotoxins [32]. Both have also been singled out on the short list of miRNAs whose expression levels are altered in association with COVID-19 [33].

It has been demonstrated experimentally that exosomes play an essential role in cell-cell communication between T-cells and B-cells during the process of antibody production following antigen presentation in germinal centers. Three specific miRNAs, one of which was miR-155, were identified as being present in these exosomes, and were essential for eliciting the appropriate B-cell response. The miRNAs promoted survival, proliferation, and antibody class switching in the B-cells, all essential for the antibody production process [34].

We have previously shown how miR-155 in particular likely plays a role in myocarditis associated with the mRNA vaccines [18]. Here, we will argue for a role for miR-146a in the induction of neurodegenerative diseases. We hypothesize that exosomes released from immune cells in the spleen travel up the vagus nerve to reach the brain stem nuclei, and they deliver their toxic cargo, which can include not only the spike protein but also intact mRNA molecules that encode the protein, to recipient cells in the brain. Microglia in the brain, in turn, could take up the spike protein and/or the mRNA, potentially leading to further upregulation of these microRNAs. miR-146a is a commonly expressed miRNA that is involved in many disease states. In particular, it is highly associated with both viral infection and prion diseases in the brain [35,36].

miR-146a has been shown to suppress rho-associated, coiled-coil-containing protein kinase 1 (ROCK1), which results in hyperphosphorylation of tau in association with Alzheimer's disease [37]. miR-146a suppresses translation of ROCK1 mRNA into protein through binding to its 3' UTR. It may be confusing that suppressing a kinase leads to increased phosphorylation of tau, but ROCK1 does not act directly on tau. ROCK1 phosphorylation of the protein phosphatase and tensin homolog (PTEN) activates PTEN to promote tau dephosphorylation. So, miR-46a suppression of ROCK1 results in PTEN inactivation which leads to the accumulation of phosphates attached to tau. Another role for ROCK1 is to repress excessive recruitment of macrophages and neutrophils during acute inflammation, so its suppression by miR-146a results in excessive macrophage and neutrophil infiltration into the tissue, thus increasing inflammation [38].

A review paper by Pogue and Lukiw states in the conclusion: "A growing body of evidence indicates that select species of the 2650-member human miRNA gene family are brain-abundant and participate in the initiation, propagation and development of insidious age-related neurological disorders of the mammalian brain and CNS. This includes the involvement of a unique pro-inflammatory miRNA-146a in a broad spectrum of viral- and prion-induced encephalopathies and related progressive age-related neurodegenerations of the human brain that include, prominently, AD [Alzheimer's disease], ALS [amyotrophic lateral sclerosis], AMD [age-related macular degeneration], MS [multiple sclerosis], TLE [temporal lobe epilepsy], scrapie and BSE (mad cow disease) as well as CJD [Creutzfeldt Jakob disease], GSS [Gerstmann-Strussler-Scheinker syndrome] and kuru."[35].

As was mentioned above, miR-146a is upregulated in response to endotoxins. The spike protein contains a sequence just above its furin cleavage site that is a superantigen-like motif sequentially and structurally similar to a segment of enterotoxin B (SEB) produced by Staphylococcus aureus [39]. Furthermore, as we will see in the next section, there is a direct signaling pathway through which it can be expected that the spike protein would upregulate miR-146a in microglia receiving the exosomes.

In a previous publication, we proposed that a major effect of the mRNA vaccines was to inhibit type I interferon signaling, leading to increasing susceptibilities to activation of latent viruses and cancer [24]. Overexpression of miR-146a could be a significant contributory factor in this downregulation. It has been shown that miR-146a suppresses type I interferon signaling, through suppression of the synthesis of Interferon Regulatory Factor 5 (IRF-5), Signal Transducer and Activator of Transcription 1 (STAT1) Interleukin-1 Receptor-Associated Kinase 1 (IRAK-1), and TNF Receptor Associated Factor 6 (TRAF6), all of which are important mediators of IFN signaling [40].

5. CD16+ Monocytes and Toll Like Receptor 4

As many as 30% of patients infected with SARS-CoV-2 continue to experience debilitating symptoms long after the virus has cleared. This condition, referred to colloquially as "long COVID," has also been formally named as "post-acute sequelae of COVID" (PASC). Common symptoms include breathlessness, fatigue, brain fog, inflammation, and coagulopathies.

A study based on 46 individuals suffering from PASC found that two specific non-classical monocyte types, (CD14Lo, CD16+) and (CD14+, CD16+) were significantly elevated in the PASC patients up to 15 months after the acute infection. A statistically significant number of these non-classical monocytes were found to still contain SARS-CoV-2 S1 protein, up to 15 months post infection [41].

A follow-on preprint study involved individuals who experienced PASC-like symptoms post vaccination for COVID-19. CD16+ monocytes were isolated from six of these patients, and it was confirmed that they too contained both S1 and S2 sequences, as well as several mutant S1 peptides [42]. It was proposed that the continual release of spike protein fragments from these monocytes could be sustaining the PASC symptoms. It is conceivable that these monocytes have reverse transcribed the mRNA into DNA, likely stored in plasmids. It has been shown experimentally that human cells expressing the retrotransposon Long interspersed nuclear element-1 (LINE-1) are able to reverse transcribe the spike protein mRNA into DNA within six hours of exposure through transfection [43].

The Gag polyprotein, present in all retroviruses, is an essential nucleic-acid-binding protein that coordinates many aspects of virion assembly, as an important step towards reverse transcription and integration into the host DNA [44]. A paper published in 2020 with the provocative title: "Prion protein PrP nucleic acid binding and mobilization implicates retroelements as the replicative component of transmissible spongiform encephalopathy," proposed that PrP is a nucleic-acid-binding antimicrobial protein that, like retroviral Gag proteins, can trigger reverse transcription by binding to LINE-1 retroelement-derived RNA. Furthermore, they claimed that PrP^{SC}'s cytotoxicity is dependent upon its ability to facilitate LINE-1 retrotransposition activity [45]. This leads to DNA double-strand breaks and cellular damage, but, as well, it can be inferred that PrP^{SC}, and, by analogy, spike protein itself, which is also an RNA-binding protein, may facilitate the retrotranscription of spike protein mRNA into DNA, mediated by LINE-1. While LINE-1 is inactive in most cells, neurons, like cancer cells and immune cells, actively express LINE-1, especially in association with neurodegenerative diseases [46,47]. The potential implications of all of this are sobering.

Fibrinogen in blood is capable of clotting into an anomalous amyloid form of fibrin that, similar to other β-rich amyloids and prions, is relatively resistant to proteolysis (fibrinolysis). A paper by DB Kell *et al.* provided evidence that the SARS-CoV-2 spike protein can interact with fibrin to form aberrant amyloid fibrin microclots, termed fibrinaloids. These microclots can inhibit the transport of erythrocytes to capillaries, disrupting the supply of oxygen to the affected tissues. They argued that this feature of the spike protein could be the primary underlying etiology of PASC [48]. In another study, when spike protein was added to whole blood, it induced platelet hyperactivation and hypercoagulation with anomalous amyloid-like clots and dense clot deposits [49]. This is strikingly

reminiscent of the ability of prion-like proteins to cause misfolding of proteins in the brain leading to neurodegenerative disease, and the underlying biophysical aspects may be analogous.

Blood monocytes recognize endotoxins produced by gram-negative bacteria through a toll like receptor 4 (TLR4) pathway [50]. The TLR4 pathway induces an inflammatory response, by upregulating both mRNA and protein levels for tumor necrosis factor α (TNF-α) and interleukin-1β (Il-1β), mediated via myeloid differentiation factor 88 (MyD88) [51]. The staphylococcal superantigen, enterotoxin B (SEB) is a potent inducer of TNF-α, and it stimulated great expansion of (CD14Lo, CD16+) monocytes. The addition of recombinant TNF-α to whole blood culture resulted in the expansion of the (CD14Lo, CD16+) monocyte population to 35% of the total monocyte pool [52]. Notably, the SARS-CoV-2 spike protein has a sequence just above the furin cleavage site that closely resembles SEB. This sequence is not present in the original SARS-CoV [39].

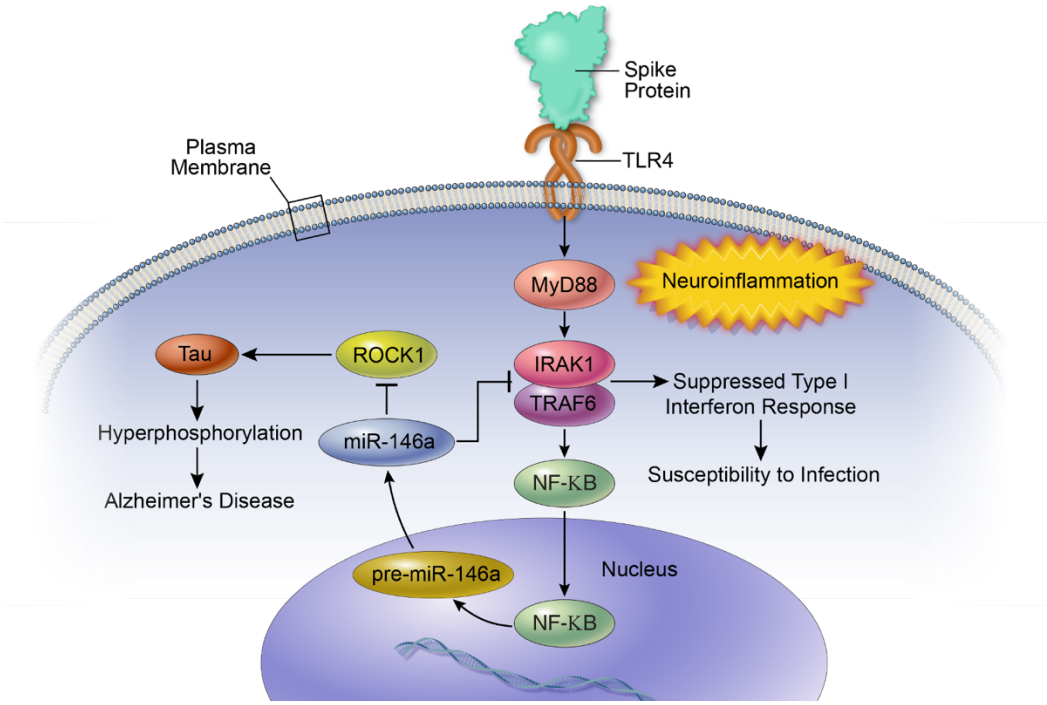

Figure 1. Schematic of pathways and consequences of spike protein binding to the TLR4 receptor in neurons and stimulating the NF-kB signaling response, leading to upregulation of miR-146a and subsequent sequelae.

Figure 1 schematizes the proposed pathways involved in spike protein activation of the TLR4 signaling response and upregulation of miR-146a in neurons. A carefully conducted experiment has demonstrated convincingly that the SARS-CoV-2 spike protein binds to TLR4 and activates it. The spike trimer directly binds to the TLR4 receptor with an affinity of ~300nM, which is comparable to the binding strength of many virus-receptor interactions. Furthermore, the spike protein robustly induces the inflammatory agent Il-1β, and this induction is lost when TLR4 inhibitors are added [53]. It is conceivable that the segment that resembles SEB is responsible for TLR4 activation.

A research paper by a team in Boulder, CO focused on the S1 subunit of the spike protein and demonstrated that injection of the S1 segment into the cisterna magna of adult male Sprague-Dawley rats resulted in behavioral deficits, microglial activation, and a neuroinflammatory response. They determined that S1 signals via a pathogen-associated molecular pattern (PAMP). In vitro experiments on transgenic TLR4 HEK293 cells showed

that S1 binds to TLR4 receptors to induce upregulation of TNF-α and other proinflammatory cytokines [54].

There is a growing body of evidence supporting a role for TLR4 in Parkinson's disease [55]. TLR4 expression is high in the substantia nigra in association with Parkinson's disease, along with upregulation of the inflammatory cytokine IL-1β [56]. Parkinson's patients also have enhanced expression of TLR4 in circulating monocytes and B cells [57].

Severe COVID symptoms share many features with sepsis [58]. The CD16+ monocyte subset is expanded in sepsis patients, and a dysregulated inflammatory response in CD16+ monoyctes is linked to sepsis [50]. Sepsis patients have elevated levels of CD16+ monocytes in their blood, which is associated with elevations in the inflammatory chemokine Il-6 [59].

The (CD14+, CD16+) monocytes have been recognized as exhibiting higher expression of proinflammatory cytokines and higher potency in antigen presentation than other monocytes, and, as such, they play a crucial role in infection and inflammation [60]. In a study on patients suffering from AIDS dementia, it was found that (CD14+, CD16+) cells represented an extremely high percentage (37% on average) of the monocytes in their blood, as compared with only 6.5% in HIV-negative controls. These authors wrote in the abstract, that these cells "might enter the brain and expose neural cells to toxic factors." [61]. Abnormally high levels of (CD14+, CD16+) monocytes are also associated with sarcoidosis [62] and complex regional pain syndrome, a condition associated with neurogenic inflammation [63].

The AIDS virus, HIV, invades the central nervous system where it causes neuroinflammation leading to cognitive impairments. A study published in 2017 demonstrated that TNF-α expression, induced by HIV, led to the shedding of PrP from astrocytes in the brain. The levels of PrP in the cerebrospinal fluid of AIDS patients suffering from cognitive issues were elevated compared to those AIDS patients without cognitive issues [64].

The S2 segment of the spike protein is responsible for membrane fusion of viral and cellular membranes. A study of the 3D structural aspects of S2 and the gp41 protein from HIV-1 revealed that these two proteins share the same two α helices and could follow an analogous membrane fusion mechanism [65]. The fact that the spike protein induces sharp TNF-α upregulation and causes cognitive issues implies that it might also, like HIV, upregulate PrP expression in the brain.

While it is unclear what the primary function of the prion protein is, it has been shown that it is protective under neuronal stress conditions. PrP expression is increased in the plasma of stroke patients, and it protects neurons from apoptosis [66]. Also, there is evidence that PrP protects cells under oxidative stress conditions from senescence. Induction of senescence in fibroblasts grown in culture through incubation with copper sulfate resulted in an increase in PrP mRNA levels, an increase in PrP protein abundance, and a nuclear localization of PrP. Knockdown of PrP expression through small interfering RNA resulted in an increase in markers of senescence. The conclusion from these findings is that PrP is upregulated under oxidative stress conditions and it helps as an antioxidant to delay senescence transformation [67].

The spike protein has been shown experimentally to induce senescence in transfected cells [68]. Macromolecular crowding can facilitate the conversion of native PrP into the neurotoxic soluble β oligomer configuration, and it is conceivable that the rapid production of spike protein from the mRNA in transfected immune cells would induce a crowded environment, while at the same time upregulating PrP synthesis due to the stressful condition [69]. This could be an ideal environment for the formation of PrP^{SC} molecules, which would be released within exosomes from transfected immune cells in the spleen and elsewhere. The transformation of PrP^{C} to the infectious PrP^{SC} molecule is an extremely slow process in the absence of PrP^{SC}. However, under the influence of intermediate PrPs, PrP^{SC} can induce solid and irreversible amyloid genesis via the template-assistance model. In another model, where PrP^{SC} is present and interacts with PrP^{C}, the progression to

amyloid genesis is fast and reversible (nuclear-polymerization model), to establish neurotoxicity [70,71].

6. A Role for Hsp70 and Diabetes

Multiple studies have shown that people who suffer from diabetes and/or obesity have an increased risk to severe outcome from COVID-19 [72,73]. One possible explanation for this observation is that these conditions disrupt the heat shock response (HSR), a natural response to a fever that normally leads to resolution of the inflammatory response [74-76]. In fact, high-risk COVID-19 patients have a suppressed anti-inflammatory heat shock response [77]. Heat shock transfer factor 1 (HSF1) is the major transcription factor regulating the expression of heat shock proteins. It suppresses the activity of Il-6 and Il-1β, thus taming the inflammatory response [78]. It is often excessive cytokine production by an overactive immune system that leads to tissue damage and life-threatening multiple-organ failure [79].

Normally, HSR induces expression of inducible heat shock protein 70 (Hsp70), also known as Hsp72 and Hspa1a, a molecular chaperone with many complex roles in metabolism and regulatory processes. Heat shock proteins can account for up to 2% of the total protein mass in a cell following activation by HSR [80]. Hsp70/72 interacts with many other proteins during the protein folding process to facilitate folding, helping to protect from the formation of protein aggregates and facilitating the degradation of damaged proteins [81].

Stressful stimuli can induce the release of intracellular heat shock proteins into the extracellular milieu and the circulation. Extracellular Hsp70/72 plays a facilitative role in the adaptive immune response to antigens [82] [60]. Also, extracellular Hsp70/72 can bind antigens, and the complex is then recognized by antigen presenting cells (APCs) via scavenger receptors. The complex is taken up by the APC, and bound Hsp70/72 protects the antigen until it reaches the proteasome. After processing, the antigen is transported to MHC class I molecules, triggering activation of cytotoxic CD8+ T-cells. The Hsp70/72-antigen complex can also be processed in the lysosome, leading to presentation of antigen-derived peptides on MHC class II molecules, thus activating CD4+ T-cells [83,84].

Impaired insulin signaling leads to a deficient ability to induce HSR and the subsequent resolution of inflammation. Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase that plays essential roles in molecular pathophysiology of many diseases. Its overexpression is linked to insulin resistance [85]. GSK-3 negatively regulates both DNA-binding and transcriptional activities of HSF1 [86]. The promoter region of the TNF-α gene contains a binding site for HSF1 that represses TNF-α transcription. Therefore, those with insulin resistance face an increased susceptibility to endotoxin exposure as a consequence of their impaired ability to induce HSF1 expression [87].

One of the most important functions of Hsp70 is to protect from neurodegenerative disease. There are many papers in the research literature linking Hsp70 to protection from various protein-misfolding neurological diseases, through its ability to facilitate proper folding and delay fibril formation [88-90]. Evidence from in vitro studies is also very clear. Pharmacological induction of Hsp70 in cells chronically infected with prions significantly decreased PrP^{SC} accumulation. Furthermore, mice lacking the gene for Hsp70 experienced accelerated prion disease progression compared with wild type mice [90].

It seems plausible that immune cells in the germinal centers in the spleen constantly synthesizing spike protein under the instruction of the mRNA in the mRNA vaccine would be under considerable stress due to the excess protein load and the potential for spike protein fragments to misfold into an amyloidogenic form. Pyrexia (fever) is a very common adverse reaction to the vaccine, indicating activation of the heat shock response. The immune cells in the spleen would be expected to upregulate Hsp70 under the influence of HSF1, and would likely release it into exosomes, along with the spike proteins and

the miRNAs such as miR-155 and miR-146a, needed to trigger an appropriate antibody response to spike. Exosomes represent a novel and efficient method for prion transmission. Stimulation of exosome release increases the intercellular transfer of prion proteins, and, conversely, pharmocological inhibition of exosome release decreases prion transfer efficiency [91]. Vaccinated obese or diabetic people would suffer from an impaired ability to instantiate the heat shock response, leaving cells taking up exosomes containing the spike protein less protected from spike protein misfolding.

7. A Potential Role for G Quadruplexes

A consideration in comparing the vaccine spike protein to the protein synthesized by the virus is related to the "codon optimization" step in specifying the mRNA for the vaccines. This practice takes advantage of the redundant nucleotide codes for most amino acids, and it involves replacing the codons used by the virus with ones that are more efficient in protein assembly. It turns out that the most efficient codons on average contain more guanines than other codons.

Guanine nucleotides, when they are enriched in the nucleotide sequence, are sometimes able to configure into a special structure called a "G quadruplex" (G4) [24]. G4s have become a hot topic in recent years due to their potential ability to regulate translation in poorly understood ways [92]. In addition, it has become apparent that the human prion protein's mRNA contains multiple G4-forming motifs, and it has been hypothesized that G4s may play a critical role in causing the prion protein to assume its misfolded state [93]. The original nucleotide sequence in the virus version of spike protein mRNA only has the potential to form four G4 motifs, whereas the Pfizer version has the potential to produce nine, and the Moderna version can form 19 [94].

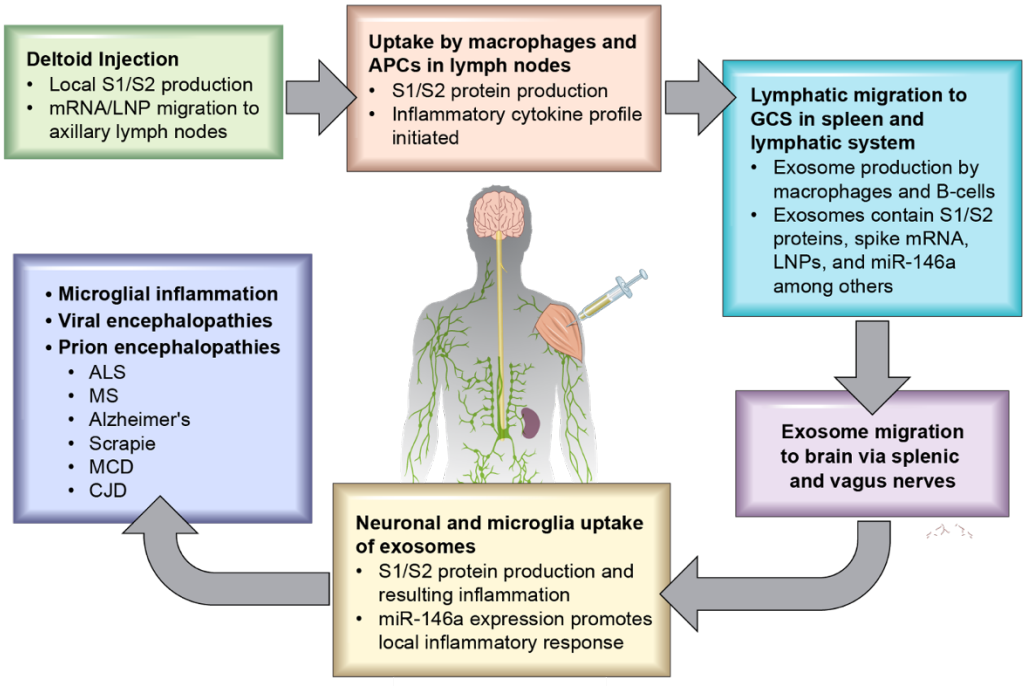

Figure 2: Schematic of the sequelae to mRNA injection into the deltoid muscle, ultimately leading to neurodegeneration in the brain. APCs = antigen presenting cells. LNPs = lipid nanoparticles. PD = Parkinson's Disease. ALS = Amyotrophic Lateral Sclerosis. AD = Alzheimer's Disease. CJD = Creutzfeldt Jakob Disease. TSE = Transmissible Spongiform Encephalopathies.

The author of a paper published in 2014, aptly titled, "G-quadruplexes within prion mRNA: the missing link in prion disease?" wrote the following in the conclusion: "The presence of G4 forming motifs in PrP mRNA may provide the missing link in the initial conversion of PrP^{C} to PrP^{SC}. Understanding how mRNA structures are involved in the (mis-)folding of PrP^{C} and possibly many other RNA-binding proteins with prion-like properties is of prime importance for the development of better treatments of CJD and related diseases." [93]

8. Conclusion

In this paper, we have examined the evidence from the extensive research literature that the SARS-CoV-2 spike glycoprotein is a neurotoxin, and that the mRNA vaccines are capable of delivering the spike protein to the brain, likely via exosomes released from the spleen, increasing the risk of neurodegenerative disease. Figure 2 shows a schematic of the likely sequence of events leading to neurodegeneration, beginning with the injection in the deltoid muscle.

Particularly concerning is the evidence that CD16+ monocytes can continuously produce spike protein for months after vaccination, possibly through reverse transcription of the mRNA into DNA. It has become clear that the antibodies induced through vaccination wane over time, necessitating frequent boosters to raise the antibody levels for sufficient protection from COVID-19. With each booster comes an increased risk of neurodegenerative disease sometime in the future. The good news is that, if the theoretical analyses are correct, the current Omicron variant has a greatly reduced prion-like capability, which may account for its observed decreased virulence.

A study published in the Lancet tracked the effectiveness of COVID-19 vaccines over time. It showed that, once eight months had elapsed since the second injection of the two-injection series, immune function was lower than that among unvaccinated individuals [95]. While boosters can temporarily restore higher levels of antibodies, frequent boosters could further erode innate immune function, for an indefinite period of time, leading to an increased risk to various infections as well as cancer. Furthermore, the rapid evolution of the virus is resulting in ever weakening antibody binding to the spike protein of the now dominant strain. Fortunately, the current strain of the virus appears to be less virulent than the original one. This may be a consequence of the decreased potential for prion-like misfolding.

In light of these considerations, the risk/benefit ratio for the mRNA vaccines needs to be reevaluated. With every vaccine comes a flood of spike protein released into the circulation, further advancing the potential for amyloidogenic effects and increasing the risk to future neurodegenerative disease. A comment by Kenji Yamamoto published in BMC is urging the medical community to keep track of the date of the most recent vaccination of hospital patients, in order to be better able to assess what role the vaccine may have played in any manifest disease or condition. He also is strongly discouraging policy that promotes continued boosting of anyone other than the most at-risk patients to death from COVID-19 [96]. There is an urgent need for governments to reconsider a blind policy that assumes that repeated vaccine boosters is a valid approach to dealing with COVID-19.

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