SARS-CoV-2 Spike Protein Impairment of Endothelial Function Does Not Impact Vaccine Safety

Technical Report

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Abstract

Lei et al. [2021] showed the spike protein in SARS-CoV-2 alone was enough to cause damage to lung vascular endothelium. The authors noted that their results suggest that "vaccination-generated antibody and/or exogenous antibody against S protein not only protects the host from SARS-CoV-2 infectivity but also inhibits S protein-imposed endothelial injury". We show that there is no known mechanism by which the spike protein impairment of endothelial function could reduce vaccine safety, and that vaccine safety data clearly shows that the spike proteins in vaccines does not reduce vaccine safety. Overall, we conclude that spike proteins encoded by vaccines are not harmful, and may be beneficial to vaccine recipients.

Keywords Vascular Biology, Vascular Disease, SARS-CoV-2, Vaccines

1 Background

COVID-19 has been widely understood to be a respiratory lung disease. However, there is now a growing consensus that SARS-CoV-2 also attacks the vascular system [Potus et al., 2020, Ackermann et al., 2020, Siddiqi et al., 2020, Teuwen et al., 2020]. Earlier studies of other coronaviruses have suggested that their spike proteins contributed to damaging vascular endothelial cells [Kuba et al., 2005].

Lei et al. [2021] created a pseudovirus surrounded by a SARS-CoV-2 crown of spike (S) proteins, but did not contain any actual virus, and found that exposure to this pseudovirus resulted in damage to the lungs and arteries of an animal model. They concluded that "S protein alone can damage vascular endothelial cells (ECs) by downregulating ACE2 and consequently inhibiting mitochondrial function".

Lei et al. [2021] noted that their conclusions suggest that vaccine-induced antibodies "not only protects the host from SARS-CoV-2 infectivity but also inhibits S protein-imposed endothelial injury". However, they did not tackle the question of whether the findings of EC damage from S protein might also have an unintended negative side effect of reducing vaccine safety.

Vaccine safety has become an important issue due to *Vaccine-induced Immune Thrombotic Thrombocytopenia* (VITT), also known as *Vaccine-induced Immune Thrombocytopenia and Thrombosis*, which has resulted in cases in recipients of the Oxford/AstraZeneca (AZ) and Johnson & Johnson (JJ) vaccines [Makris et al., 2021]. VITT refers to a rare combination of thrombosis (usually CVST) and thrombocytopenia which have been found in some patients 4 to 30 days after they receive their first AZ or JJ vaccine dose (and occasionally after their second dose).

Regulators have found that clots are extremely rare, and that the benefits of the vaccines outweigh the risks. However, the roll-out of the AZ and JJ vaccines have been restricted in many jurisdictions [Mahase, 2021]. In the UK, for instance, the Joint Committee on Vaccination and Immunisation (JCVI) recommend avoiding the AZ vaccine for

those under 40 years old, based on "reports of blood clotting cases in people who also had low levels of platelets in the UK, following the use of Oxford/AstraZeneca vaccine." [Public Health England, 2021]

With an Altmetric Attention Score of 3726 (as of May 23rd, 2021), Lei et al. [2021] has become the most discussed paper in the history of Circulation Research, and is in the top 0.005% of discussed papers across all topics. By reading a random sample of social media posts that link to the paper, we found that the great majority of readers express a view that the paper shows that the vaccine is not safe, and that therefore people should not get vaccinated. This view has also been widely shared in blog posts, such as Adams [2021], which states "Bombshell Salk Institute science paper reveals the covid spike protein is whats causing deadly blood clots and its in all the covid vaccines (by design)", and concludes "The vaccines literally inject people with the very substance that kills them. This isn't medicine; its medical violence against humanity". Furthermore, some doctors are now publicly expressing concerns about vaccine safety, based on concerns about the impact of spike proteins. [Bruno et al., 2021]

Because Lei et al. [2021] did not explicitly discuss the relevance of its findings to vaccine safety, and because it has been widely cited as showing that vaccines are not safe, including by some doctors, we will examine whether or not its findings should result in pausing or stopping the vaccine roll-outs.

2 Analysis of Current Data

To ascertain whether spike protein impairment of endothelial function reduces vaccine safety we can directly observe the results of vaccine use.

2.1 Overall Vaccine Safety

The vaccine with the most recorded cases of VITT is the AZ vaccine. The largest roll-out of the AZ vaccine is in England. The roll-out began in December 2020, and by the start of February 2021 over 10 million people had received at least one dose. By mid-April 2021, over 10 million people had received their second dose.

Public Health England publishes data on "Excess mortality in England". This data shows that from March 20, 2020, until February 19, 2021, there were 101,486 excess deaths in England. From February 20, 2021 (two months after the start of England's vaccine roll-out), until April 30, 2021 (the latest data available at writing), there have been no excess deaths in England.

As of May 5, 2021, there were 262 reported cases of VITT in the UK after the first dose of the vaccine, resulting in 51 deaths, and eight cases have been reported after a second dose [Medicines & Healthcare products Regulatory Agency, 2021]. 35 million people had received their first vaccination by this time. This is over half the population of the UK, and nearly all adults over 30 years old. Children and young adults in the UK will not be receiving the AZ vaccine, based on current guidelines.

Overall, with 51 deaths due to VITT, compared to 101,486 probably due to COVID-19, we can see that the overall impact of the vaccine is to greatly reduce deaths. Even if the spike proteins in vaccines resulted in reduced endothelial function (which, as we shall see shortly, they do not), the impact would clearly not be significant enough to result in the need to reduce or stop vaccine roll-outs.

2.2 Comparative Analysis

All currently approved SARS-CoV-2 vaccines incorporate spike proteins. If the spike proteins in vaccines resulted in significantly reduced endothelial function, causing VITT, then we would expect to see reports of VITT in recipients of all the available vaccines.

However, this is not the case. There are no reports of VITT in recipients of the Moderna or Pfizer vaccines.

It is unlikely that this is due to failure to identify VITT, since the particular combination of thrombosis and thrombocytopenia is very rare, and the issues around vaccine safety widely reported and discussed.

Furthermore, it is statistically unlikely. As of May 15, 2021, in the USA 156.2 million people had received at least one dose of SARS-CoV-2 vaccine, the vast majority of which were Pfizer and Moderna. Since each recipient's vaccine VITT response is an independent binary event, we can model it with a binomial distribution. The UK VITT death rate is 0.0001%. If the spike proteins were the cause of VITT, we would expect the same death rate in the US, which would result in 183-273 deaths (99% confidence interval). However, we have seen zero reports of VITT in the US.

3 Mechanism of genetically-encoded spike protein vaccines

Lei et al. [2021] found that freely circulating, spike protein-decorated pseudovirus at a very high dosage (half a billion pseudovirus particles per animal) delivered directly to the trachea damages lung arterial endothelial cells in an animal model. Similarly, an extremely high concentration (4 micrograms per milliliter) of purified recombinant spike protein could damage human pulmonary arterial endothelial cells *in vitro* [Lei et al., 2021]. These extremely high concentrations were used to simulate what may happen during a severe case of COVID-19 infection, wherein humans may have what some have estimated to be as high as 1 to 100 billion virions in the lungs [Sender et al., 2020]. Given there are approximately 100 spike proteins per virion [Neuman et al., 2011], this means COVID-19 infections could in theory result in as many as 10 trillion spike proteins. In wild-type viruses, the spike protein is cleaved such that the S1 portion is released and can be free to circulate in the serum [Xia et al., 2020], where it could potentially interact with ACE2 receptors on the endothelium. Thus, in both the spike protein laboratory experiments described in Lei et al. [2021] and in severe COVID-19 cases, exceedingly large amounts of freely circulating spike protein are present.

Animal studies have been performed to measure the distribution of genetically-encoded vaccines and their protein products. In the intramuscular injection site, which is by definition where the maximum amount of payload (i.e. lipid nanoparticles-packed mRNA or adenovirus) will be present and, by extension, where the maximum amount of spike protein will be produced, the payload is undetectable within 24-72 hours *in vivo* and the protein is undetectable within 10 days at most, and closer to 4 days post-injection when using lower doses more similar to that given to patients. Animal studies show there is some dispersal of payload to distal regions of the body, but as expected the concentrations dramatically decrease from maximum concentration at the injection site (5,680 ng/mL) to much lower concentrations elsewhere, for example they found >3000x lower concentrations (1.82 ng/mL) in the lung, and 10,000x lower concentrations in the brain (0.429 ng/mL) [Feldman et al., 2019].

Given only a fraction of the payload will be expressed, and given that the measurements of mRNA do not necessarily distinguish between functional, full-length mRNA versus non-functional mRNA fragments, only a small fraction of the measured mRNA will be translated into spike protein. The distribution of actual spike protein throughout the body appears to follow an even steeper gradient — *in vivo* luciferase measurements in animals treated with mRNA vaccines show significant protein expression almost entirely confined to the site of injection [Pardi et al., 2015]. Note that the concentration given to patients is even lower than those used in these animal studies, and that the dispersion appears to drop off faster for lower doses. Overall, these data indicate relatively low, transient amounts of spike protein are produced by the vaccine, and the vast majority of spike protein produced is confined to the site of injection. Therefore, the concentration of freely circulating spike protein from vaccines available to the public is bound to be many orders of magnitude times lower than the amount used in Lei et al. [2021]. The impairment found in that study would not be expected from the relatively tiny, physiologically irrelevant amount of spike proteins found in a vaccine.

In order to be physiologically relevant to, let alone damaging to blood vessels, freely moving, soluble spike proteins would have to enter the circulatory system at high enough concentrations to bind and disrupt a significant number of ACE2 receptors on a significant number of vascular endothelial cells. As discussed above, measurements indicate that no significant amount of vaccine enters circulation. The confinement of the expressed spike protein away from the circulatory system prevents it from causing significant damage. In addition to the confined localization of expression, there is another safeguard preventing spike protein from accessing the vascular endothelium in any significant amount: The vaccine uses an engineered form of the spike protein that is fused to a transmembrane anchor. The transmembrane anchor allows the spike protein to appear on the surface or membrane of the cell, but it is held in place by the anchor. This prevents the vast majority of spike protein from drifting away while at the same time creates a fixed target for the immune system to recognize and develop antibodies against the spike protein [Corbett et al., 2020]. While there is a chance for the mRNA-expressing cells to release full spike protein upon destruction by immune cells, the amount released is only going to be a small fraction of that produced by the vaccine, and certainly at too low a level to be physiologically relevant.

In agreement with the mechanism-based estimates outlined above, Ogata et al. [2021] recently published empirical measurements of freely circulating spike protein produced by the vaccines using an ultra-sensitive SIMOA assay. Their measurements revealed the average spike protein levels to be less than 50 picograms per milliliter, approximately 100,000x lower concentration than that used in Lei et al. [2021]. These femtomolar levels are far too low to be physiologically relevant, let alone pathological. Importantly, peak spike protein levels are reached within days after injection, and rapidly disappear to undetectable levels within 9 days of the first injection, and much lower to undetectable levels within 3 days after the second injection. At the same time, antibodies against spike protein are inversely correlated with circulating spike protein, supporting the hypothesis that anti-spike antibodies can quickly and effectively neutralize freely circulating spike protein.

4 Adenovirus Vector-Based Vaccines and VITT

Importantly, the endothelial damage described by Lei et al. [2021] is not the mechanism by which VITT occurs. VITT is an extremely rare and unique form of adverse event associated only with adenovirus vector-based vaccines. It is not caused by spike proteins targeting the endothelial cells, but rather due to induction of an immune response against platelet factor 4 (PF4) by adenovirus vector-based vaccines. PF4 is released by platelets and causes them to clump and form small clots and have a physiological role in stopping bleeding (hemostasis). Antibodies are not generated against self PF4, but have been described as a rare side effect of heparin, a commonly used blood thinner. In this condition termed heparin inducted thrombocytopenia (HIT), heparin binds to PF4 and the complex then stimulates an aberrant immune response. Antibodies to PF4 are generated, and these antibodies bind to PF4, and the resulting immune complex then binds to platelets and activates them. This releases more PF4, and a cycle ensues. Activated platelets in HIT form arterial and venous clots, and as platelets get consumed in the clots, the platelet count drops resulting in severe thrombocytopenia. This combination of clots and severely low platelets is unusual.

The reason VITT raised an alarm even with very few cases was the unique HIT like clinical presentation in the absence of any heparin exposure. The seriousness of the condition, and the need to use a blood thinner besides heparin also made it an important clinical and management issue. Studies now show that in VITT, the adenovirus vector-based vaccine is able to induce high levels of PF4 antibodies in 1 in 100,000 to 1 in 500,000 individuals much the same way as heparin does in patients with HIT [Greinacher et al., 2021a].

Greinacher et al. [2021b] assessed the clinical and laboratory features of VITT patients, and found that the AZ vaccine "can result in the rare development of immune thrombotic thrombocytopenia mediated by platelet-activating antibodies against PF4". The AZ vaccine contains the preservative EDTA, which can help human cell-derived proteins from the vaccine enter the bloodstream, binding to PF4 and producing antibodies Greinacher et al. [2021a]. Lab tests showed that "High-titer anti-PF4 antibodies activate platelets and induce neutrophil activation and NETs [neutrophil extracellular traps] formation, fueling the VITT prothrombotic response" Greinacher et al. [2021a]. Although the JJ vaccine does not use EDTA, it is an adenovirus vector-based vaccine, which is a particularly inflammatory stimulating virus [Appledorn et al., 2008, S Ahi et al., 2011]. The lack of EDTA may result in less cases of VITT, but even without EDTA proteins from the vaccine can enter the bloodstream.

The hypothesis that the cause of VITT is due to acute inflammatory reactions to vaccine components independent of the spike protein in adenovirus vector-based vaccines is consistent both with the experimental results of Greinacher et al. [2021a], and is consistent with the observation that only the AZ and JJ vaccines (both of which are adenovirus vector-based vaccines) have been associated with VITT, whereas the Moderna and Pfizer vaccines (both of which are mRNA vaccines) have not been associated with VITT.

Although the hypothesis of Greinacher et al. [2021a] has not yet been fully confirmed, it is consistent with lab testing, empirical evidence, the extreme rarity of VITT, and mechanistic constraints. It is also possible that it is remediable, since it is not due to the nature of the vaccine itself, but specific to the particulars of the formulation.

5 Conclusion

Given these observations, we conclude the vaccines do not produce enough freely circulating spike protein to induce vascular damage via the ACE2 receptor destabilization mechanism described in Lei et al. [2021]. On the contrary, the extremely low, femtomolar levels of circulating spike protein induced by the vaccine are unlikely to have any physiological relevance to vascular endothelial cells, while still allowing the immune system to develop a robust immune response to spike proteins. The presence of anti-spike antibodies may in fact serve to protect vaccinated individuals against not only SARS-CoV-2 infection, but also against spike-protein induced damage to the vascular endothelium. We speculate that this protection against spike protein-induced damage may in part explain why COVID-19 symptoms are much less severe in vaccinated individuals Rossman et al. [2021].

There is now a very large amount of empirical data available that clearly shows the benefits of all approved SARS-CoV-2 vaccines are far greater than the risks of extremely rare side effects. The data also is not consistent with the hypothesis that VITT is due to spike proteins, since the Pfizer and Moderna vaccines are not resulting in any reports of VITT. The data is, however, consistent with the hypothesis that side effects are due to inflammatory reactions to vaccine components in adenovirus vector-based vaccines.

Overall, we conclude that all approved SARS-CoV-2 vaccines provide far more benefits than risks, and that the very rare risk of VITT from the AZ and JJ vaccines is not due to the spike proteins, which are a fundamental part of how the vaccines work, but is most likely due to specific details of the formulation of the vaccines.

6 Competing interests:

No competing interests declared.

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