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Letter

# The spike hypothesis in vaccine-induced adverse effects: questions and answers

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**Trougakos and colleagues recently discussed the role of coronavirus disease 2019 (COVID-19) mRNA vaccine-induced spike (S) protein in adverse effects following vaccination [1]. We would like hereafter to answer one of their outstanding questions requiring response to improve efficacy and safety of COVID-19 vaccines, and to propose an additional question along with its possible answer.**

## Does the S protein leak into the circulation, at what concentration, and for how long?

Trougakos and colleagues [1] appropriately point to the crucial importance of measuring vaccine-induced S protein in blood and tissues and its time course. Systemic bioavailability of COVID-19 mRNA vaccines has been indeed excluded until May 2021, when circulating S1 subunit was reported as early as 1 day after the first injection of mRNA-1273 COVID-19 vaccine, up to 150 pg/ml and for about 2 weeks after injection [2]. The S1 subunit estimated molecular weight is 90–100 kDa [3], one mole would therefore be 90 000–100 000 g and the 150 pg/ml plasma concentration reported in [2] would be 1.5 fmol/ml or 1.5 pM. Such a concentration attained in plasma is lower than the S1–ACE2 receptor equilibrium dissociation constant, which has been reported to be about 120 nM [4]. The equilibrium dissociation constant measures the propensity for the bound ligand/target complex to dissociate to free ligand and target, and corresponds to the ligand concentration that is necessary to bind 50% of the available target. Regarding the S1–ACE2 receptor complex, plasma concentrations 100 000-fold lower than the equilibrium dissociation constant as in this case (1.5 pM vs 120 nM), likely exclude any significant S protein binding to ACE2 receptors. Circulating S protein following vaccination, however, originates from endogenous production, and its concentration is therefore likely higher in tissues where production occurs. For example, levels of the neurotransmitter dopamine are up to 100 million times higher in brain areas where it is produced, in comparison to plasma where it occurs as a result of tissue spillover [5]. It should be investigated whether the same occurs for COVID-19 vaccine-induced S protein, eventually leading to potentially toxic concentrations in tissues and organs where S protein happens to be produced. Indeed, in a woman with mRNA-1273 COVID-19 vaccine-induced thrombocytopenia, plasma S protein levels 10 days after vaccination were 10 ng/ml [6], thus nearly 100 times higher than those reported by Ogata and colleagues in vaccinated subjects with no apparent adverse effects [2], pointing to possibly excessive vaccine-induced production of S protein, in turn attaining concentrations high enough to significantly bind targets such as ACE2, and eventually resulting in vaccine toxicity.

Circumstantial evidence also suggests that endogenous production of S protein following vaccination may occur for a long time. Both vaccine mRNA and the S protein have been detected in axillary lymph nodes up to 60 days after the second dose of mRNA-1273 or BNT162b2 COVID-19 vaccines [7], and at least one preprint study claims to have identified the S protein in blood samples by means of proteomic analysis up to >6 months after mRNA vaccine administration [8]. Would vaccine-induced S protein production occur for such a long time, then the plausible time window for causality assessment of suspect adverse reactions following COVID-19 mRNA vaccines should be suitably extended.

## How does production of S protein compare between COVID-19 mRNA vaccines and SARS-CoV-2 infection?

During COVID-19, circulating S1 subunit was detected in most patients, with median levels about 50 pg/ml and maximum levels about 1 ng/ml, possibly as a result of viral antigen leakage into the blood in subjects with severe disease, as also suggested by correlations between higher S1 with intensive care unit admission and time to intubation [9]. Such levels are comparable to those reported after COVID-19 mRNA vaccination (up to 150 pg/ml) [2] and lower than those measured during vaccine-induced thrombocytopenia (10 ng/ml) [6], thus raising the possibility that severe infection and vaccination, eventually result in similar total systemic amounts of S protein.

Regarding infection, a recent theoretical calculation suggests 1–100 billion SARS-CoV-2 virions occurring in an infected person [10]; thus, since individual virions express on average 24 ± 9 S protein trimers [11], the total amount of S proteins could be 24 × 3 × 1–100 billion = 72–7200 billion.

Concerning vaccines, S protein amounts produced by COVID-19 mRNA vaccination could be obtained from the time course of plasma levels after vaccine injection [2]. Let us take for example data from subject #1 (Supplementary Figure 2 in [2]) (for detailed calculations, please see Box 1):

day 0 – 0 pg/ml

day 5 – 55 pg/ml

day 7 – 70 pg/ml

Box 1. Calculation of S protein amounts produced by COVID-19 mRNA vaccination

Total amount of SARS-CoV-2 S1 protein production following vaccination was estimated using data from subject #1 (Supplementary Figure 2 in [2]):

day 0 – 0 pg/ml

day 5 – 55 pg/ml

day 7 – 70 pg/ml

day 9 – 25 pg/ml

day 14 – 0 pg/ml

Let us use the following formula:

$Q = AUC \times CL$

where Q is the total amount, AUC is the area under the curve of plasma levels over time, and CL is the clearance of S1 protein, which is taken from [12] based on S1 molecular weight and set at 0.01 ml/min or 14.4 ml/day.

AUC was calculated by means of the trapezoidal rule:

$55\ pg/ml \times 5\ d : 2 = 137.5\ pg \times d/ml$

$(55\ pg/ml + 70\ pg/ml) \times 2\ d : 2 = 125\ pg \times d/ml$

$(70\ pg/ml + 25\ pg/ml) \times 2\ d : 2 = 47.5\ pg \times d/ml$

$25\ pg/ml \times 5\ d : 2 = 62.5\ pg \times d/ml$

thus in total: 372.5 pg × d/mL = AUC

Therefore $Q = AUC \times CL = 372.5\ pg \times d/mL \times 14.4\ mL/d = 5364\ pg = 5.4\ ng$

Since 1 M of S1 protein is 100 000 g, then 5.4 ng = 0.9 billion molecules.

day 9 – 25 pg/ml

day 14 – 0 pg/ml

The total amount (Q) would be:

$Q = AUC \times CL$

where AUC is the area under the curve of plasma levels over time, provided that the clearance (CL) of the S1 protein is known, which unfortunately, is not the case. Nonetheless, since CL of peptides is often related to their molecular weight [12], and the S1 molecular weight is 90–100 kDa [4], a likely estimation of S1 CL might be 0.01 ml/min or 14.4 ml/day, and the total amount of S1 produced by subject #1 would be then about 5.4 ng, corresponding to 0.9 billion S1 protein, an amount which is slightly lower but nonetheless comparable to the lower limit of the estimated production during COVID-19 (72 billion; [10]). Nevertheless, such comparison should be examined, also taking into account the 100-times higher plasma levels measured in the patient with mRNA-1273 COVID-19 vaccine-induced thrombocytopenia mentioned above [4] (which suggests higher vaccine-induced S protein production when severe adverse effects occur), as well as considering that most of the virus-derived S protein likely remains in the respiratory tract, while vaccine-induced S protein production occurs in internal organs and tissues (thus being in the position to exert more systemic effects).

## Concluding remarks and future perspectives

Such considerations as a whole support the possibility that COVID-19 mRNA vaccines under some circumstances induce high and possibly toxic amounts of S protein in organs and tissues, in turn leaking into the circulation. In animal models, it is well established that lipid nanoparticle-carried mRNAs undergo systemic disposition and expression in organs such as liver, skeletal muscle, and lungs [13]. It can be suggested that at least part of the risk to develop adverse reactions following vaccination with mRNA products depends on the organs/tissues where S protein production occurs, as well as on the total amount produced and on the production time course. For example, it was established long ago that distinct tissues widely differ in the efficiency of protein synthesis, but no one so far assessed whether and to what extent this is relevant for the efficacy and safety of mRNA vaccines [13]. Therefore, we further recommend careful characterization of COVID-19 vaccines with regards to systemic disposition, including organs and tissues where S protein production occurs and interindividual variability in local protein synthesis efficiency, to provide a rational basis for dose individualization and to identify subjects at risk for adverse reactions due to either vaccine-induced S protein production in vulnerable sites, excessive S protein production, or both [14].

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