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Commentary

SARS-CoV-2 immunogenicity: Is S protein the best target for vaccination?

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1. Introduction

The large numbers of individuals of almost all ages that received vaccines based on SARS-CoV-2 spike(S)-protein mRNA/DNA constructs facilitated the study of the on- and off-target toxicity. Despite the importance of S protein in SARS-CoV-2 infectivity, its highly regulated functions mislead scientists towards viral benefit. High fucosytation using host's enzyme machinery correlates to production of afucosylated IgG1, which upon binding to activating FcγRIIIa receptors leads to inflammation. The engagement of angiotensin converting enzyme 2 (ACE2) with S protein, which is one of the central mechanisms for the viral entry to host's cells, decreases ACE2 availability, affecting thus many important ACE2-regulated pathways, while also relating to off-target side-effects of the S protein-based vaccines. In addition, S1 sub-unit of the S protein seems to inhibit fibrinolysis, leading to ischemic conditions. Since multiple antigenic epitopes are recognized by CD4/CD8-positive T cells in various HLA class I and II allotypes, it is probably time to consider other than S protein targets for prophylactic vaccinations.

2. Multiple protein targets for SARS-CoV-2 vaccine development

SARS-CoV-2 has an approximately 30 kb in length genome and almost 80% sequence identity with SARS-CoV. The genomic RNA comprises 2 main open reading frames (ORFs), namely ORF1a and ORF1b, which cover 2/3 of the genome and are translated to polyproteins 1a and 1ab that upon cleavage by viral proteases produce nonstructural proteins (Nsps), Nsp1 to Nsp16 (Table 1) [1]. The remaining 1/3 of the genome encodes four structural proteins: spike (S), membrane (M), envelope (E) and nucleocapsid (N), and some accessory proteins including ORF3a, ORF6, ORF7a, ORF7b, ORF8, ORF9 and ORF10 (Table 1).

Although S protein has been chosen as the target protein in most vaccine applications, immunogenicity analyses of SARS-CoV-2-derived HLA class I and class II T cell epitopes demonstrate that other structural, non-structural and accessory proteins are also recognized and may become the target in infected individuals. Thus, Grifoni et al. [2], using HLA class I and II predicted peptide pools, showed that SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells could be identified in 100% and ~70% of 20 COVID-19 patients, respectively, where $CD4^+$ T cell responses to M, S and N proteins accounted for 11–27% of the total $CD4^+$ responses, with additional responses targeting among others nsp3, nsp4, ORF3a, and ORF8, while $CD8^+$ T cell responses to S and M along with at least eight different targeted ORFs were recognized. Interestingly, the authors had noticed that ~40–60% of unexposed individuals bear SARS-CoV-2-reactive $CD4^+$ T cells. Similarly, Nelde et al, [3] based on integration algorithms identified SARS-CoV-2-derived HLA class I- and HLA-DR-binding peptides across all ten viral ORFs and performed predictions for the ten and six most common HLA class I (HLA-A*01:01, -A*02:01, -A*03:01, -A*11:01, -A*24:02, -B*07:02, -B*08:01, -B*15:01, -B*40:01 and -C*07:02) and HLA-DR (HLA-DRB1*01:01, -DRB1*03:01, -DRB1*04:01, -DRB1*07:01, -DRB1*11:01 and -DRB1*15:01) allotypes in 180 infected individuals, respectively. In most cases the authors detected major preferences for N, M, ORF1 and ORF3 proteins in the case of class I allotypes and mainly N proteins in the case of HLA-DR allotypes.

3. Spike protein biology

Spike (S) protein is a homo-trimer, each monomer consisting of 1273 residues and contains two major sub-units namely S1, which recognizes the human angiotensin converting enzyme 2 (ACE2) receptor, and S2, which mediates fusion to the host's cell membrane [1]. The monomeric S protein contains 22 N-linked glycosylations (66 N-glycan sequins within the trimer) and 2O-glycosylation sites at the receptor-binding domain of subunit S1. Hijacking the glycosylation machinery of the host cells, viruses succeed to camouflage immunogenic epitopes, thereby enhancing evasion to the host's immune response. Cryo-electron microscopy analysis of trimer surface shielded from antibody recognition by glycans showed that high mannose glycans shielded trimer surface by 44%, paucimannose by 31%, biantennary complex type glycans by 43% and core-fucosylated biantennary complex types by 45% [4]. Using a site-specific mass spectrometric approach, Watanabe et al. [5] showed that across the 22 N-linked glycosylation sites,

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Table 1
Description and role/function of SARS-CoV-2 proteins.

	Protein role/Function
Non-structural proteins	
Nsp1	Degradation of host mRNA, inhibition of interferon signaling
Nsp2	Unknown
Nsp3	Cleavage of viral polyprotein, de-ADP-ribosylation, de-ubiquitination, interferon antagonist, double-membrane vesicle formation
Nsp4	double-membrane vesicle formation
Nsp5	Cleavage of viral polyprotein, inhibition of interferon signaling
Nsp6	double-membrane vesicle formation
Nsp7	Co-factor for RNA-dependent RNA polymerase
Nsp8	Primase for 3′-terminal adenylyltransferase, Co-factor for RNA-dependent RNA polymerase
Nsp9	Single-stranded RNA binding
Nsp10	Co-factor for nsp14 and 16
Nsp11	Unknown
Nsp12	RNA-dependent RNA polymerase, nucleotidyltransferase
Nsp13	Helicase, RNA 5′ triphosphatase
Nsp14	3′ to 5′ exoribonuclease, proofreading RNA cap formation, guanosine N7-methyltransferase for viral RNAs
Nsp15	Endoribonuclease, evasion of immune response by preventing detection of viral dsRNA by the host
Nsp16	RNA cap formation, ribose 2′-O-methyltransferase
Structural proteins	
Spike (S)	S1 sub-unit: binds to host's receptor, S2 sub-unit: mediates viral and host membrane fusion
Membrane (M)	Organizes viral assembly
Envelope (E)	Interacts with M to form viral membrane
Nucleocapsid (N)	Binds genomic RNA forming nucleocapsid
Accessory proteins	
ORF3a	Activates inflammasome
ORF6	Type I interferon antagonist
ORF7a	Induces apoptosis
ORF7b	RNA-dependent RNA polymerase, nucleotidyltransferase
ORF8	Down-regulates MHC-I in cells
ORF9	Suppresses type I interferon activity
ORF10	unknown

52% are fucosylated and 15% of the glycans contained at least one sialic acid residue. Among the different glycosylation sites, N343 was shown to be highly fucosylated with 98% of detected glycans bearing fucose residues [5].

Core-fucosylation is an essential biological modification by which a fucose is transferred from GDP-β-L-fucose to the innermost N-acetylglucosamine residue of N-linked glycans. A single human enzyme α1,6-fucosyltransferase (FUT8) is the only enzyme

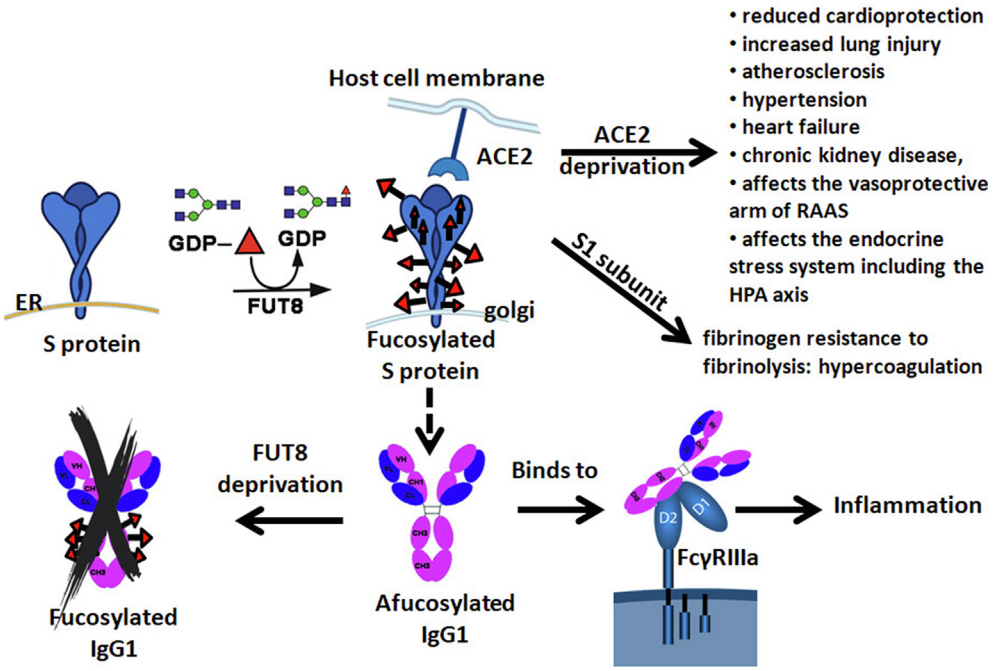

Fig. 1. Harmful activities of the S protein to the host. Deprivation of FUT8 disallows fucosylation of IgG1, which in the afucosylated form binds to the activating FcγRIIIa receptors leading to inflammation. Deprivation of ACE2 leads to a number of pathologies because of the deregulation of the renin-angiotensin system, the RAAS and the endocrine system. The S1 unit itself induces fibrinogen resistance to fibrinolysis contributing to hypercoagulation.

that catalyzes this modification via the addition of an α-1,6-linkage. Human IgG molecules contain two N-glycans in the CH2 domains of their Fc region, where in the case of IgG1, the core oligosaccharide consists of GlcNAc (core fucose)-GlcNAc-mannose-(mannose- GlcNAc)2. IgG is a highly core fucosylated glycoprotein, and the lack of core fucosylation increases the binding affinity to FcγRs, enhancing the antibody-dependent cell mediated cytotoxicity (Fig. 1) [6]. Patients with severe COVID-19 produced a unique serologic signature, including an increased likelihood of IgG1 with afucosylated Fc glycans. This Fc modification on SARS-CoV-2 IgGs enhanced interactions with the activating FcγRIIIa receptor, leading to production of inflammatory cytokines by monocytes [7].

Since fucosylation is solely catalyzed by FUT8, it seems that S protein fucosylation deprives IgG from using such type of glycosylation, describing thus an additional mechanism of SARS-CoV-2 attack. When S protein is provided to the organism as a nucleic acid vaccine, such mechanisms cannot be excluded, and could therefore account for some side-effects of these vaccines (Fig. 1).

4. Spike protein ACE2 receptor

Another important reason for considering that S protein is not the right target for vaccine development is its engagement with ACE2 receptor. Such interaction exhausts ACE2 from the organism and disregulates the renin-angiotensin system leading to reduced cardioprotection, increased lung injury, contributing to atherosclerosis, hypertension, heart failure, chronic kidney disease [8], while also affecting the vasoprotective arm of the rennin-angiotensin-aldosterone system (RAAS) as well as the endocrine stress system, including the hypothalamic–pituitaryadrenal (HPA) axis (Fig. 1) [9]. ACE2 is a zinc metalloprotease that is expressed in the respiratory tract, lung, ileum, bladder, esophagus, heart, kidney, hypothalamus, pituitary and adrenal and controls the generation of the angiotensin 1–7 from angiotensin II, as well as angiotensin 1–9 from angiotensin I, which is thereafter also converted to angiotensin 1–7 by ACE1. The yin/yong relationship of ACE1 and ACE2 keeps the equilibrium in the renin-angiotensin signaling. ACE1 generates angiotensin II and signaling by the angiotensin (AT)1 and AT2 receptors, while ACE2 generates angiotensin 1–7 whose receptor (MAS) opposes responses mediated by AT1. AT1 overexpression is related to all pathologic conditions mentioned above. Indeed, ACE2, angiotensin 1–7 and MAS receptor constitute the vasoprotective arm of the RAAS, whose extensive activation leads to arterial hypertension and immune-metabolic disorders. Furthermore, ACE2 is also related to stress response and anxiety through the control of corticosterone and proopiomelanocortin expression in plasma and pituitary, respectively. The involvement of ACE2 in the HPA axis correlates with the elevated stress levels of SARS infected individuals [9]. Although HPA dysfunction was recovered one year after infection, psychiatric morbidities and chronic fatigue persisted and continued to be clinically significant up to at least 4 years after the SARS outbreak [9]. Such observations are not yet available for SARS-CoV-2 infected individuals.

5. Spike protein in coagulation

Finally, the S1 subunit of the S protein itself seems to induce fibrinogen resistance to fibrinolysis contributing thus to hypercoagulation in COVID-19 patients [10] (Fig. 1). Using scanning electron and fluorescence microscopy as well as mass spectrometry, it was shown that S1 subunit induces structural changes to β and γ fibrinogen, complement 3, and prothrombin, which become resistant to trypsinization. Such property of the S1 subunit cannot be excluded in the use of S protein-based vaccines.

6. Conclusion

A number of recent findings highlight the problems that S protein may cause to the organism and discourage its use as target for vaccine development, while also explaining the observed side-effects of S protein-based vaccinations. The large numbers of immunized individuals have provided the opportunity to define the on and off-target toxicity of mRNA and DNA vaccines in a short period of time. The extensive analyses of CD4 and CD8 specificities as well as HLA class I and II susceptibilities in SARS-CoV-2 infected individuals point out additional viral targets, which will also need to be extensively tested.

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CRediT authorship contribution statement

Tania Antonopoulou: Conceptualization. **Irene Athanassakis:** Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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