EDITORIAL

Be aware of SARS-CoV-2 spike protein: There is more than meets the eye

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The COVID-19 pandemic necessitated the rapid production of vaccines aimed at the production of neutralizing antibodies against the COVID-19 spike protein required for the corona virus binding to target cells. The best well-known vaccines have utilized either mRNA or an adenovirus vector to direct human cells to produce the spike protein against which the body produces mostly neutralizing antibodies. However, recent reports have raised some skepticism as to the biologic actions of the spike protein and the types of antibodies produced. One paper reported that certain antibodies in the blood of infected patients appear to change the shape of the spike protein so as to make it more likely to bind to cells, while other papers showed that the spike protein by itself (without being part of the corona virus) can damage endothelial cells and disrupt the blood-brain barrier. These findings may be even more relevant to the pathogenesis of long-COVID syndrome that may affect as many as 50% of those infected with SARS-CoV-2. In COVID-19, a response to oxidative stress is required by increasing anti-oxidant molecules. In this regard, it is known that polyphenols are natural anti-oxidants with with anti-inflammatory properties. Hence, there are even more reasons to intervene with the use of anti-oxidant compounds, such as luteolin, in addition to available vaccines and anti-inflammatory drugs to prevent the harmful actions of the spike protein.

Infection with the recent Coronavirus [severe acute respiratory syndrome (SARS)-CoV2] leads to COVID-19, the severity of which derives from the host's immune response (1), especially the release of a storm (2, 3) of pro-inflammatory cytokines (4-10) and prothrombotic molecules, such as platelet activating factor (PAF), leading to microemboli (11, 12).

The SARS-CoV-2 coronavirus infects cells by first binding to its surface receptor, Angiotensin Converting Enzyme 2 (ACE2), via its corona spike protein (13, 14). The spike protein is made up of the S1 subunit containing a receptor-binding domain (RBD) that attaches to ACE2 and the S2 subunit containing a transmembrane anchor that mediates fusion of viral and host cell membranes. A number of vaccines for COVID-19 were developed rapidly using either mRNA or adenovirus vector technology aimed at directing cells to produce the spike protein so that the body will generate neutralizing antibodies (15, 16). However, recent papers have reported intriguing, but also disturbing, findings concerning detrimental actions of the spike protein (Table I).

One paper still in preprint stage at Cell reported that certain antibodies in the blood of patients infected

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Table I. *Detrimental effects of spike protein*

• SARS-CoV-2 entry into target cells
• Endothelial damage
• Proinflammatory cytokine release
• TLR activation
• Microglia stimulation
• Molecular mimicry with chaperon and heat shock proteins

with SARS-CoV-2 appear to change the shape of the spike protein so as to make it more likely to bind to cells and infect them. Evidently, antibodies against the RBD are protective, but antibodies against the N-terminal domain (NTD) induced the open conformation of the RBD enhancing the binding ability and infectivity (https://www.cell.com/cell/pdf/S0092-8674(21)006620.pdf). Another paper reported that the spike protein shares antigenic epitopes with human molecular chaperons resulting in autoimmunity against endothelial cells (17). In fact, the spike protein by itself (without being part of the corona virus) was shown to damage endothelium in an animal model via impaired mitochondrial function (18). A fourth paper reported that the spike protein could alter barrier function in an in-vitro model of the blood-brain barrier (BBB); in particular, the S1 protein promoted loss of barrier ability in an advanced 3D microfluidic model of the human BBB (19). Finally, S1 protein was reported to actually cross the BBB and enter the brain of mice (20), possibly leading to neuroinflammation (21). In fact, another recent study reported blood vessel damage and inflammation, but no infection, in brains of patients with COVID-19 (22).

These findings may help explain the pathogenesis of COVID-associated neurological (23-26) and mental (27-31) symptoms, especially "brain fog" (32, 33). Moreover, these results could be central in our understanding of Long-COVID syndrome (34, 35) that may affect over 50% of COVID patients (32, 36, 37) and is characterized by neurologic (38) and psychiatric (39) symptoms (32, 34, 35, 40), as well as persistent fatigue apparently independent of the severity of the initial symptoms (41). Symptoms

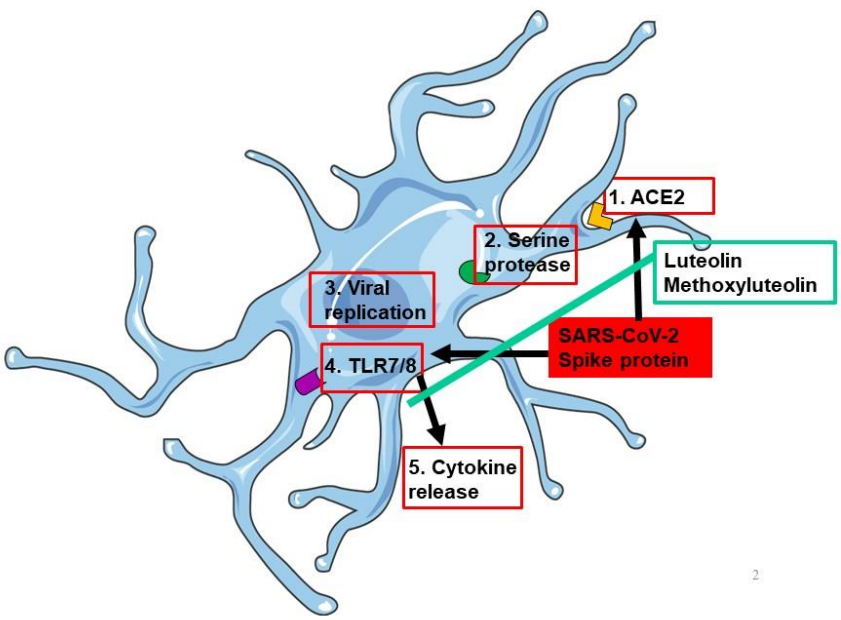

Fig. 1. *Diagrammatic representation of how luteolin and methoxyluteolin could block SARS-CoV-2 Spike protein from stimulating microglia. The biologic action of SARS-CoV-2 Spike protein could be via different steps (red rectangles): (1) Spike protein binding to its ACE2 receptor; (2) Activation of serine proteinases responsible for "priming" the Spike protein for entry into the cells; (3) Viral replication within the nucleus; (4) Activation of TLR7/8 found in the endosomes by single-stranded RNA viruses like SARS-CoV-2; (5) Production of proinflammatory cytokines. Luteolin and methoxyluteolin could protect against SARS-CoV-2 Spike protein-associated damage by interfering (green line) at practically all steps.*

experienced by Long-COVID syndrome patients are similar (42, 43) to those present in patients with Mast Cell Activation Syndrome (MCAS) (44, 45).

Emerging evidence suggests mast cell activation or a dysfunctional response play a role in COVID-19 via the release of multiple pro-inflammatory cytokines (1, 2, 4-10) and prothrombotic mediators (11, 12), especially since mast cells are stimulated by viruses (46). The result is inflammation around brain blood vessels (22) that may generate a vicious cycle or autoimmune loop of neuroimmune processes casing L-COVID. In fact, preliminary reports suggest that mast cells may be involved in COVID-19 (47-49) and that individuals taking substances interfering with the mast cells or their mediators are less likely to suffer severe COVID-19 (33, 43). COVID-19 can also affect the hypothalamic-pituitary-adrenal (HPA) axis (50), which is typically activated by stress (51), but also has pro-inflammatory effects via stimulation of mast cells by corticotropin-releasing hormone (CRH) (53). Mast cells can interact with microglia in the brain, leading to their activation with subsequent neuroinflammation (52-54) and cognitive dysfunction (55). Microglia express Toll-like receptors (TLRs) (56), activated by damage associated molecular patterns (DAMPs) and were recently implicated in COVID-19 (57-60).

Given the above, it is reasonable to consider preventing the detrimental actions of the spike protein (Table I). This could be accomplished by the use of liposomal formulations (61) of the natural flavonoids luteolin and methoxyluteolin (62-64), which are safe (65, 66) and have broad anti-viral properties (67) by acting at different steps (Fig. 1): Spike protein binding to its ACE2 receptor; 2) Activation of serine proteinases responsible for “priming” the Spike protein for entry into the cells; 3) Viral replication within the nucleus; 4) Activation of TLR7/8 found in the endosomes by single-stranded RNA viruses like SARS-CoV-2; 5) Production of proinflammatory cytokines.

Furthermore, luteolin and methoxyluteolin better penetrate into the brain, inhibiting both microglia (68-70) and mast cells (71, 72), while they also reduce neuroinflammation (66, 73, 74) and cognitive dysfunction (75, 76), especially brain fog (77, 78).

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