Review Article

Toxicity of SARS-CoV-2 Spike Protein from the Virus and Produced from COVID-19 mRNA or Adenoviral DNA Vaccines

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Abstract

COVID-19 has already killed millions of people around the globe through severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection followed by immuno-inflammation processes induced by viral proteins in particular the Spike protein. COVID-19 vaccines in particular mRNA and DNA vaccines have been proposed and used worldwide but they have been recognized to be associated with or being the cause of various side effects. Generally mild but also more severe side effects have been observed such as thrombosis, myocarditis, hepatitis, neurological side effects, and menstruation perturbations which are accessible and described in official registries (Vaccine Adverse Event Reporting System (VAERS-CDC, EudraVigilance). The purpose of this article is to summarize and discuss immuno-inflammatory and other biochemical mechanisms that may be common to both COVID-19 pathology and vaccination side effects using mRNA and DNA technologies which induce the human body's production of a close replica of the spike protein of SARS-CoV-2. We propose that the main inflammatory pathway may be the activation of the complement system (lectin and alternate pathways). Others key toxic pathways are the activation of [des-Arg9] -bradykinin (ACE2/bradykinin B1R/DABK axis), dysregulation of the renin-angiotensin-aldosterone system as well as the attenuation of the Mas receptor activity (ACE2/MasR axis). Also the crucial importance of oxidative stress associated to inflammatory processes has been largely underestimated. Altogether this review indicates tracks for additional research efforts to identify more efficient and precise anti-inflammatory/antioxidant treatments against both COVID-19 and COVID-19 vaccine side effects.

Keywords: Spike protein - COVID-19 Vaccines side effects – Immuno-Inflammation – Lectin pathway – Bradykinin – renin angiotensin aldosterone

MeSH Keywords: Spike protein - SARS-CoV-2 - COVID-19 - COVID-19 vaccine - Inflammation – Blood - Coagulation - Microglia

Introduction

The spike protein allows SARS-CoV-2 to anchor itself onto human cells via its receptor Angiotensin-converting enzyme 2 (ACE2) which is a membrane-bound peptidase [1]. Subsequently, the spike protein is cleaved by the transmembrane protease serine 2 (TMPRSS2) to mediate virus fusion with the cell membrane and its uptake [2]. ACE2 is a key protein for our health. Indeed it is an enzyme which has crucial roles for the human organism: it helps to regulate blood pressure through the renin angiotensin aldosterone system (RAAS), and it is essential to fight inflammation in our body, which is

particularly key in the pathology of COVID-19. When ACE2 is occupied by its link with the virus, it can no longer play its essential anti-inflammatory role. In particular that induces an imbalance with another enzyme with which ACE2 is normally in equilibrium, the Angiotensin-converting enzyme (ACE), which activates inflammation and which is left free by the virus. This ACE2/ACE imbalance participates to the hyper- inflammation in COVID-19 that develops through major biochemical pathways described in this review. By binding to ACE2 the spike protein of the virus triggers a significant part of the inflammation, the so called "cytokine storm", pneumonia and potential acute respiratory distress syndrome (ARDS) with clotting, and finally death. It is this very closely the same spike protein that mRNA and DNA vaccines produce in large and uncontrolled quantities in the body and that may have similar affinity for ACE2 receptor [3, 4]. Therefore, the spike protein from the vaccines in particular in its free form, is potentially able to trigger the same inflammatory processes as the virus spike protein. The inflammatory toxicity of spike protein has been reported and well known in the scientific literature for at least ten years. The second factor which increases the toxicity of spike/ ACE2 interaction is that ACE2 receptors are present almost everywhere in the body: pharynx, trachea, lungs, blood, heart, vessels, intestines, brain, male genitalia and kidneys, and also in body fluids (mucus, saliva, urine, cerebrospinal fluid, semen and breast milk) [5].
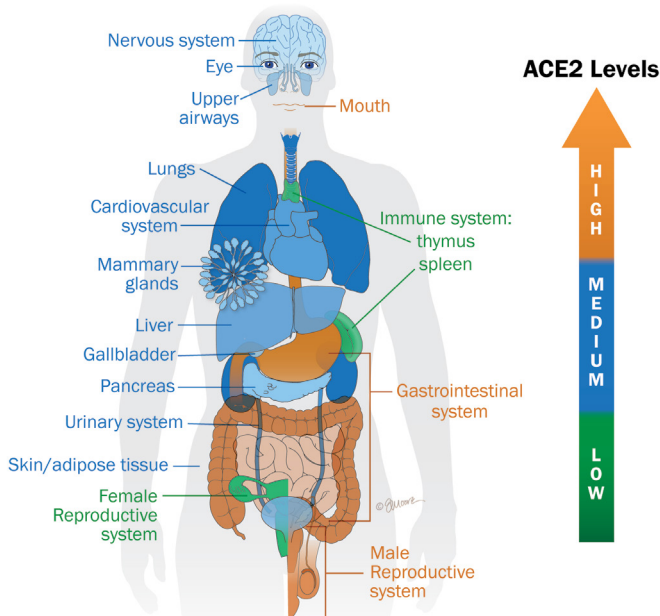
As a result, spike protein is able to set off inflammation in many organs and systems. Indeed, the majority of patients with COVID-19 have various other symptoms in addition to respiratory disorders, including neurological, cardiovascular, intestinal and renal dysfunctions [6, 7, 8, 9, 10]. Since spike protein is present in SARS-CoV-2 (and its variants) and produced by the vaccines, this toxicity therefore applies both to COVID-19 (severe forms and also long COVID) but also potentially to all vaccines which are based on the uncontrolled production of this spike protein by various cells versus vaccines which are made from deactivated whole virus or based on deactivated spike protein. It is known that after injection with mRNA vaccines, spike protein is found both on the surface of the cells (where the manufacturers have told it would be found) but also in significant quantities under free form circulating in blood thus reaching different organs including the heart, brain, liver, kidneys, and the brain [11]. Moreover, it has been demonstrated that the whole spike protein alone as well as the S1, which contains the ACE-2 receptor-binding domain (RBD), can interact with ACE2 receptors expressed by various cells, including platelets and endothelial cells, inducing inflammatory processes [11,12]. Therefore, it is imperative that the vaccine companies give complete information not only on the toxicity of mRNA encapsulated in lipid nanoparticles (LNPs) but also of vaccine spike protein.

Description of spike protein-induced immuno-inflammatory processes and associated bio-chemical pathways

The main hallmark of severe cases of COVID-19 is pneumonia, with potential acute respiratory distress syndrome (ARDS) that requires patients to be treated in intensive care unit. After the replication phase of the virus a strong inflammation occurs. Fifteen percent of patients develop dysfunctional and harmful procoagulant and inflammatory disease, which results in life-threatening respiratory distress. The famous "cytokine storm" is an uncontrolled release of cytokines that has been observed in some infectious and non-infectious diseases. But the term is often used, including by many physicians and researchers, without sufficiently specifying the biochemical pathways involved which impacts negatively the search of more precise potential therapies. The spike protein allows great dissemination of the virus through the epithelia and then through the bloodstream to various organs. This is also the case for the vaccines. In these organs and vessels, spike proteins can spread inflammation by the 4 main following pathways (figure 2):

1. Dysregulation of the renin-angiotensin-aldosterone system (ACE/angiotensin II / AT1R axis)

2. Attenuation of the Mas receptor activity (ACE2/MasR axis)

Figure 1: Overview of the expression level of ACE2 receptors

The color gradient from orange to green indicates high or low level of ACE2 expression in tissues and body fluids. The highest levels are detected in the oral cavity, gastrointestinal tract and male reproductive system [5].

3. Activation of [des-Arg9] -bradykinin (ACE2/bradykinin B1R/DABK axis)

4. Activation of the complement system (lectin and alternate pathways) including components C5a and C5b-9

Dysregulation of the renin-angiotensin-aldosterone system (ACE/angiotensin II / AT1R axis)

The renin-angiotensin-aldosterone system (RAAS) is, in mammals, one of the most important regulatory systems for autonomic, cardiovascular, and pulmonary functions. It is a hormonal system organized around the kidney, which makes it possible to preserve hydrosodium homeostasis. The RAAS regulates blood pressure and also plays an important role in inflammation [14]. As mentioned previously, ACE2 protein plays a beneficial role: vasodilator, antioxidant, and anti-inflammatory while its counterpart, the ACE protein is on the contrary vasoconstrictor, pro-oxidant, and pro-inflammatory. ACE2 plays this role by controlling the amount of an essential peptide, angiotensin II produced by ACE from another small protein or peptide, angiotensin I. Angiotensin I is produced by the kidney as a result of the enzyme renin from the angiotensinogenic peptide which is formed in the liver and released into the plasma. As ACE2 is depleted because of the presence and multiplication of the virus, the balance tilts in favor of the inflammation produced by angiotensin II which is no longer degraded through the numerous mediators that result from it especially in the lungs. This inflammation occurs through the interaction between angiotensin II and the AT1 receptor (Angiotensin II type 1 receptor, AT1R) in the kidney and the vascular system [15, 16]. A significant production of cytokines results from this, such as the growth factor TGF-β. Angiotensin II can then activate the pivotal pathway of nuclear factor kappa B (NF-κB), a central component of the inflammatory response, which is also associated and induced by other important cytokines in COVID-19: IL-1B , IL-6, TNFα, IL-10, MCP1 (monocyte chemoattractant protein 1), AT1 and platelet-derived growth factor beta (PDGF-B) [17, 18, 19].

Attenuation of the Mas receptor activity (ACE2/MasR axis)

The ACE2/MasR receptors and Ang- (1–7) are the constituents of the other arm of the RAAS system which counteracts and attenuates the effects of the first axis ACE-Ang II-AT1R described above and illustrated in Figure 3 [20].

Angiotensinogen is secreted by the liver and is converted to angiotensin I (AngI) via renin, a protease produced in the kidneys. AngI is then converted to AngII by the catalytic action of angiotensin converting enzyme (ACE) and binds to angiotensin II type 1 (AT1) and type 2 (AT2) receptors. AngII binds to the angiotensin type 1 receptor (AT1R) to promote actions such as vasoconstriction, cell proliferation, fibrosis, and inflammation. ACE2 converts Ang-I and Ang-II to angiotensin (1–7). Ang (1–7) binds to the MAS receptor (MASR) to promote vasodilation, vascular protection, anti-fibrosis, anti-proliferation, anti-inflammation and anti-angiogenesis actions [20].
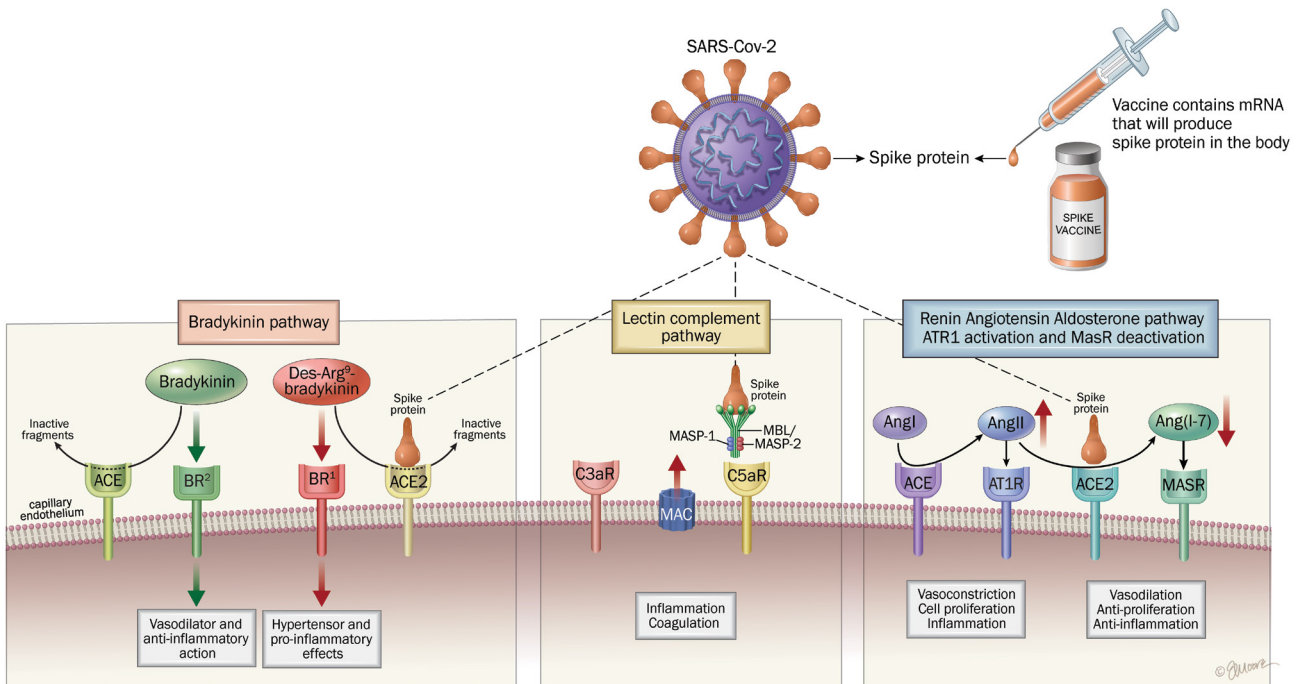

Figure 2: Main inflammatory pathways explaining the toxicity of SARS-CoV-2 spike protein with respiratory infection and produced from COVID-19 mRNA and adenoviral DNA vaccines.

This is an axis of protection against inflammation which is weakened due to the monopolization of the ACE2 receptor by the virus. The beneficial effect of ACE2 occurs through the cleavage of angiotensin II into the Ang- (1–7) peptide. It is the latter molecule which has a vasodilator effect, antiproliferative (anti-cancer), anti-thrombotic and anti-inflammatory properties and is therefore found to be in deficit. Ang- (1–7) reduces the expression of inflammatory factors such as NF-κB, IL-6, TNFα and IL-8 [21, 22, 23].

Activation of [des-Arg9] -bradykinin (ACE2/brady-kinin B1R/DABK axis)

Infection with SARS-CoV-2 by depleting ACE2 also increases the levels of des-Arg9-bradykinin (DABK) which is a known pulmonary inflammatory peptide factor. DABK is a bioactive metabolite of bradykinin which is associated with lung damage and inflammation, primarily through bradykinin receptor 1 (BR1) receptors in the endothelial cells of the lungs. The kinin-kallikrein system includes kininogen, the enzyme kallikrein, bradykinin (BK-1–9 or BK) and DABK (figure 4). ACE2 participates in the degradation of DABK, a process ultimately by the downregulation of ACE2 induced by the virus and thus leading to a loss of control of this inflammatory pathway [24]. Crosstalk between angiotensin, the kinin-kallikrein system, as well as the inflammatory and coagulation systems play a critical role in COVID-19. Cardiac complications and coagulopathies involve crosses between these systems and should be a more tested route of study to prevent or control ARDS in patients with severe COVID-19 [25, 26, 27].

A review of the literature suggests that the control of the bradykinin pathway may be a feasible option in the management of COVID-19 in patients with vascular pathologies and that a supportive treatment of respiratory complications and cardiology is necessary in COVID-19 patients, in particular by C1-inhibitors (C1-INH) [26, 28, 29]. This inflammatory pathway appears to be much more important in COVID-19 than the first two described.
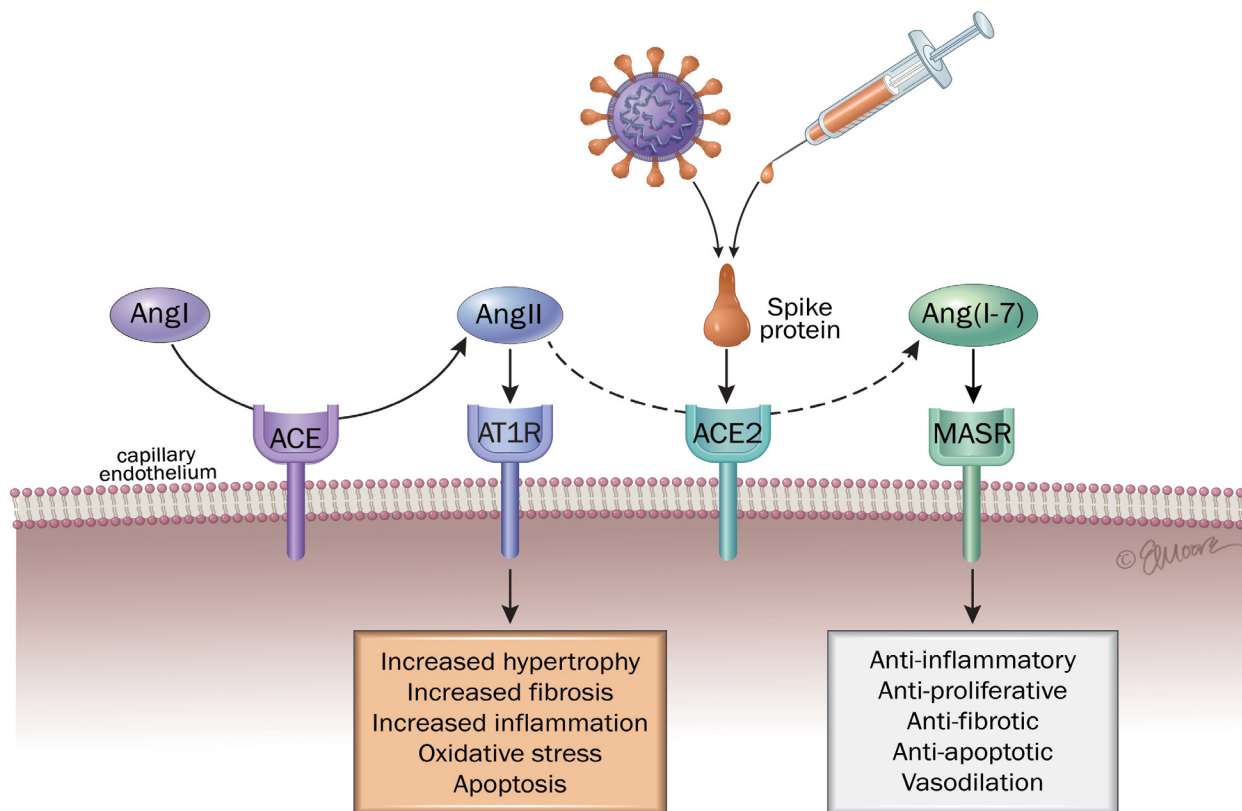
Figure 3: Schematic overview of RAAS perturbation by spike protein (virus or vaccine spike)

Angiotensinogen is secreted by the liver and is converted to angiotensin I (AngI) via renin, a protease produced in the kidneys. AngI is then converted to AngII by the catalytic action of angiotensin converting enzyme (ACE) and binds to angiotensin II type 1 (AT1) and type 2 (AT2) receptors. AngII binds to the angiotensin type 1 receptor (AT1R) to promote actions such as vasoconstriction, cell proliferation, fibrosis, and inflammation. ACE2 converts Ang-I and Ang-II to angiotensin (1–7). Ang (1–7) binds to the MAS receptor (MASR) to promote vasodilation, vascular protection, anti-fibrosis, anti-proliferation, anti-inflammation and anti-angiogenesis actions [20].

Figure 4: Action of ACE and ACE2 enzymes in the KKK system (kininogen-kinin-kallikrein)

A. ACE degrades bradykinin, a vasodilator peptide acting primarily through BR2 receptors. Bradykinin can also be converted by kininase I to des-Arg9-bradykinin (DABK), which promotes vasoconstriction and pro-inflammatory effects upon interaction with BR1 receptors.

B. SARS-CoV2 mediated imbalance in the KKK system with predominant pro-inflammatory effect of des-Arg9-bradykinin [27].

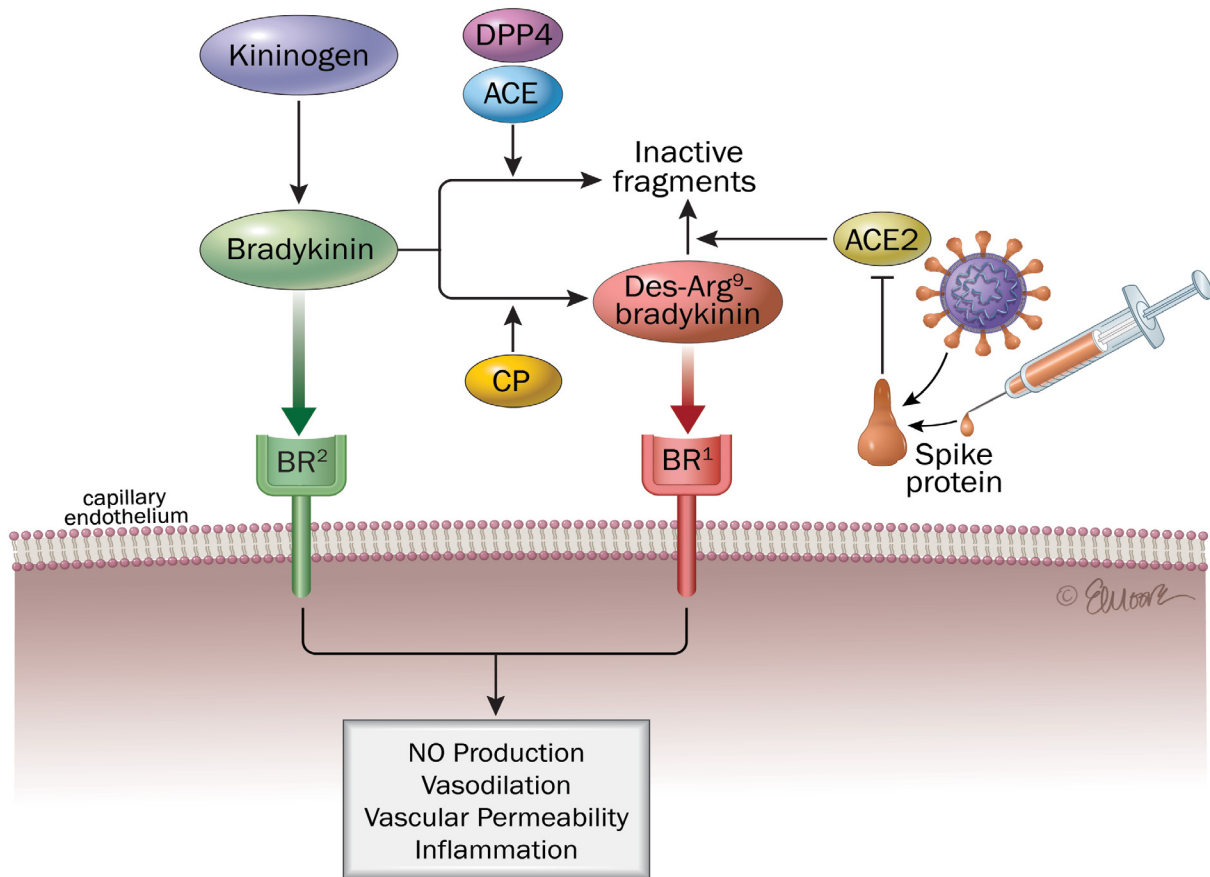## Activation of the complement system (lectin and alternate pathways) including components C5a and C5b-9

The complement system contributes to the innate immune response which includes the cells and mechanisms immediately allowing the body's defense against infectious agents in the absence of antibodies. It does not require cell division, conversely of the adaptive immune system which confers a later but more durable protection and which requires cell division (lymphocytes B and T). Its excessive activation during COVID-19 is involved in cytokine storm, endothelial inflammation, and the thrombosis that accompany the disease in the most severe forms and death.

There are three biochemical pathways that activate the complement system: the classical complement pathway, the alternate complement pathway, and the lectins pathway binding mannose and N-acetylglucosamine residues of the sugars present in bacterial and viral membranes. The involvement of the complement system in the pathogenesis of infection by syncytial virus, MERS-CoV and SARS-CoV-1, is already known from several studies [30, 31, 32]. Hyperactivation of complement components includes C5a in serum and C5b-9 in lungs was observed in hDPP4 transgenic mice infected with MERS-CoV. Lung and spleen induced damage and inflammatory responses were attenuated by blocking the C5a - C5aR axis in these transgenic mice [33].

The lectin pathway is a priority pathway in COVID-19 [32]. It is activated by the binding of a mannan-binding lectin (MBL)-mannan-binding lectin serine protease 2 (MASP-2) complex to the surface of a pathogen. This complex, linked to the pathogen, then cleaves C4 and C2. These cleavages form a C3 convertase (C4b2a). Studies and literature reviews highlight the role of MASP binding to SARS-COV-2, activation of the lectin pathway, and blood coagulation, as well as their associations with COVID-19 comorbidities. Therefore, it is likely a key pathway linking

inflammation and the end-stage coagulation phenomena of severe forms of COVID-19. Although N protein activates the lectin complement pathway, the spike protein has also been shown to activate this pathway [34, 35, 36, 37]. Of important note, different molecules of the biochemical lectins pathway were found in the lung tissues recovered during autopsies of subjects who were reported to die of COVID-19: MBL, MASP-2, C4alpha, C3 or C5b- antibody 9 [30].

Lectin-type receptors (CLRs), in particular dendritic cell-specific intercellular adhesion molecule-3-Grabbing non-integrin (DC-SIGN) (and also liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin [L-SIGN], mannose receptor [MR], and macrophage galactose lectin [MGL]), on the surface of cells of the innate immune system (such as macrophages and dendritic cells) can direct immune responses of the host against the virus (S proteins in particular) by identifying specific sugars (glycans: oligomannose N-glycans) associated with virus proteins [38].

Viral inactivation by the complement cascade involves absorption and clearance of the virus by phagocytosis, which prevents attachment to their receptors, lysis of the virus by

the formation of pores, and destruction of its membrane by formation of a membrane attack complex (C5b-9) (MAC) [39]. The increase in Ang II production and the activation of AT1R are accompanied by a pro-inflammatory response via the activation of the complement cascade comprising C5a, and C5b-9. Complementary factor 5a (C5a) is the most powerful inflammatory peptide in the complement cascade which induces the release of numerous pro-inflammatory cytokines including IL-1, IL-6, TNF-α, NF-κB, activator Protein-1 (AP-1) [40]. In addition, increased production of C3a leads to the production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α. If the virus has not been cleared by the immune system at the epithelial barrier, it will then progress to vascular endothelium which express ACE2 receptors.

Viral penetration into these cells will cause endothelial inflammation called endothelitis or endothelialitis [41]. Endothelitis is associated with the thrombotic phenomena observed in COVID-19, such as microthrombosis and macrovascular thrombosis, as well as pulmonary embolism [42, 43]. The complement has been associated with
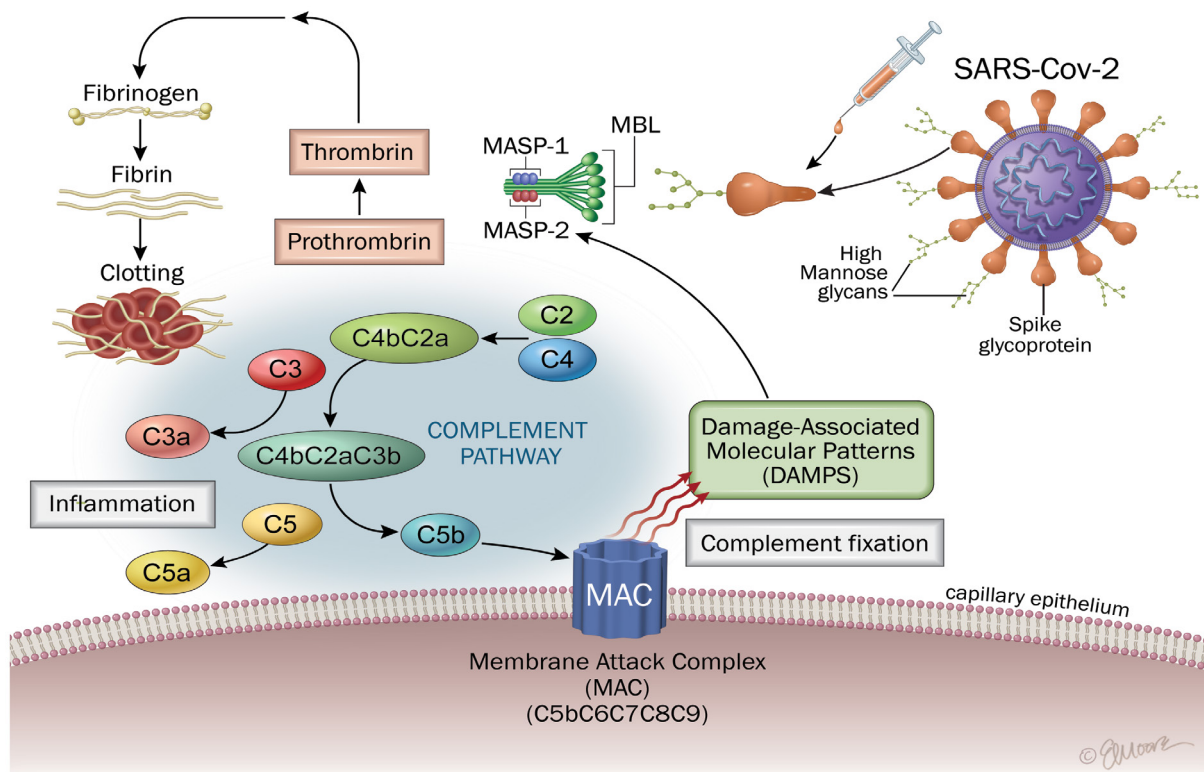
Figure 5: Lectin-complement pathway induced SARS-CoV2 and mRNA vaccines spike protein

The lectin pathway is activated by the binding of an MBL (mannan-binding lectin)-MASP-2 (mannan-binding lectin serine protease 2) complex to the surface of a pathogen. This complex, linked to the pathogen, then cleaves the C4 and C2. These cleavages form a C3 convertase (C4b2a). Activation of the classical pathway is initiated by binding of the C1 complex to immunoglobulins or endogenous ligands. This complex will be able to cleave C4 and C2 to form the classic C3 convertase (C4b2a) which cleaves the C3 into C3a, an anaphylatoxin, and into C3b, causing the formation of C5 convertase (C4b2a3b or C3bBb3b). The C5 is then cleaved into C5a and C5b, which will initiate the final stages of the complement cascade. This leads to formation of the membrane attack complex (C5b-9, MAC) which allows pathogens to be lysed.

endothelitis lesions seen in severe forms of COVID-19. Histological deposits of MASP-2, C4 and MAC, as well as macrophages overexpressing the C5a receptor, C5aR1, have in fact been found in endothelitis lesions and microthrombi [44]. The complement and coagulation (contact phase, or intrinsic pathway of coagulation) are closely linked [45]. Indeed, MASP-1 and MASP-2 have a catalytic role for prothrombin and fibrinogen, leading to thrombotic complications (figure 5) [46, 47]. Finally, MASP 1 and/or 2 have increased expression in patients with risk factors for COVID-19: diabetes, high blood pressure and cardiovascular disease, chronic kidney disease, chronic obstructive pulmonary disease and cerebrovascular disease [33].

A comparison of spike protein-induced toxicity in the setting of SARS-CoV-2 infection versus injections of COVID-19 mRNA and adenoviral DNA vaccines. Proposal of biochemical mechanisms

As mentioned before, during COVID-19, the immuno-inflammatory pathways occur not only in lung, but also in all organs that contain ACE2 receptors [5]. This is also potentially the case for the spike protein generated in high amounts in the body and of which a part has been found to circulate free in the circulation [11]. Therefore, it is justified to investigate more deeply and to propose mechanisms for both COVID-19 disease and vaccine side effects in all the organs where they have been reported. Severe side effects are difficult to assess and there is currently no consensus on the exact counts. Of note, the US VAERS database provide a signal that appears significant and sometimes associated mortality which may be largely underestimated. Indeed, it is estimated that the reports of side effects represent only about 10% of reality [48].

Damage on vessels and cardiovascular system

Lei et al. (2021) showed that using a pseudo-virus expressing S protein (pseudo-spike devoid of viral RNA), specifically the S1 part which contains the RBD of SARS-CoV2 caused inflammation and damage in the arteries and lungs of mice exposed intratracheally [49]. The same phenomenon has been observed on human epithelial cells resulting in attack of the mitochondria. The work of these researchers clearly shows that the spike protein alone, not associated with the rest of the viral genome, is sufficient to cause the cardiovascular damage associated with COVID-19. This suggests that both SARS-CoV-2 and free spike released after COVID-19 vaccination could result in the same cardiovascular damages. Moreover, in an *in vitro* study, researchers showed that spike alone (S1 part) induced a loss of integrity of the blood-brain barrier (which separates the vessels supplying the brain from the central nervous system) on a model reconstructing this barrier, suggesting

the possibility of inflammation in brain vessels [50]. For vaccines, in addition to the free spike found in the circulation, the spike protein can be expressed on endothelial cells and can activate blood platelets and coagulation, which also leads to thrombosis (by releasing platelet factor 4 or PF4) [51, 52].

Thrombosis after vaccination have been described, as well as myocarditis and pericarditis, stroke, in association high levels of D-dimer (coagulation marker) and CRP (C-reactive protein: inflammation marker) [52, 53, 54]. Of note, in a post-vaccination study with the AstraZeneca vaccine, thromboses were observed on young subjects (between 22 and 49 years of age, mean age 36 years) whereas severe COVID-19 occur essentially in much older people and elderly [52]. They developed venous thromboses, including nine patients with cerebral thrombosis, three with pulmonary embolism and six of the eleven patients died. When circulating virus and spike proteins for vaccine react with the ubiquitous ACE2 receptors in the body this upset the ACE2/ACE balance at these sites [3, 55]. It is known that in almost all pathological conditions, in particular those involving the cardiovascular system but also neurodegenerative damages, there is a decrease in the ACE2/ACE ratio within the organs [56, 57, 58, 59, 60].

Some cases of sudden cardiac deaths have also been described. The same four mechanisms highlighted in this review (including RAAS, the complement pathway and bradykinin) could be partly implicated, but we also propose here the hypothesis that a rupture of the cardiac syncytium could occur especially after various injections. As it has been proposed and shown for a few COVID-19 cases, spike protein could drive cell-cell membrane fusion between cardiomyocytes that would lead to aberrant electrophysiological activity, electromechanical dysfunction, and fatal arrhythmia [61, 62]. In a young woman who died suddenly and was diagnosed post-mortem with COVID-19, some authors have observed highly focal myocardial SARS-CoV-2 infection spreading from one cardiomyocyte to another, through intercellular junctions identified by highly concentrated sarcolemmal t-tubule viral spike glycoprotein [61].

The formation of syncytial multinucleated giant cells facilitated by S2 spike domain could lead ultimately to irreversible electrophysiological dysfunction and sudden cardiac arrest [63, 64, 65]. In the case of vaccines it could be facilitated by the presence of LNPs [66].

Another hypothesis could be the formation of amyloid structure. It has been proposed that severe inflammatory disease including ARDS in combination with SARS-CoV-2 protein aggregation might induce systemic AA amyloidosis [67]. Cases which showed amyloid deposits in vessels shortly after vaccination have been described [68]. Furthermore several sequences of spike protein of around 20 amino-acids (192−211, 601−620, 1166−1185) fulfill amyloid fibril criteria

[69]. Neutrophil elastase which is overexpressed on sites of inflammation of viral infection, cleaved spike protein, thus permitting the exposure of amyloidogenic segments.

Neurological toxicity and damages

SARS-CoV-2 is not only a respiratory virus but is in fact multi-target. In particular, it has a high tropism for the central nervous system (CNS) as shown by several previous studies [70, 71, 72]. A study covering 214 COVID-19 patients, reported that 78 (36.4%) had neurological manifestations which included malaise, headache, and loss of smell (anosmia) and taste (dysgueusia) as well as more serious complications, such as ischemic stroke associated with significantly higher mortality [70, 73]. SARS-CoV-2 is also detected in the brain of a majority of examined patients [71]. ACE2 receptors are expressed in the human CNS, particularly in the spinal cord, spinal ganglion, brainstem nigra, choroid plexuses, hypothalamus, hippocampus, middle temporal gyrus, and posterior cingulate cortex [74, 75].

The virus has been shown to pass through olfactory or vagus nerve, as well as the blood–brain barrier (BBB) [70, 74, 76]. Another route is the blood-cerebrospinal fluid (CSF) barrier, which is a more permeable barrier compared to the BBB, formed by a single layer of endothelial cells of the choroid plexus [77, 78]. Inflammation induced by SARS-CoV-2 infection in the CNS seem to be linked to microglia activation and astrocytes inflammation [79]. Microglial activation has been observed in several studies including post-mortem studies [71, 80, 81]. After vaccination spike protein could also initiate inflammation in CNS through microglia and astrocytes, as well as the mechanisms described before (lectin-complement, bradykinin, and RAAS). It has been shown that mRNA and free spike protein can reach the brain through circulation and by crossing the BBB [50, 82].

Bahl et al. showed that mRNA of vaccines can reach the brain, as also specified by the European Medicines Agency (EMA) up to 2% of the mRNA plasma concentration [83, 84]. Other authors have shown that S1-induced neuro-inflammation in microglia mediated by the activation of NF-κB and p38 MAPK and increased release of TNF-α, IL-6 and IL-1β and NO [85]. Spike alone (S1 part) induced a loss of integrity of BBB on a model reconstructing this barrier, suggesting the possibility of inflammation in brain vessels and in the brain itself [50, 82]. Furthermore, activated microglia as well as activation of complement represent a common pathological feature of several neurodegenerative diseases, including Alzheimer's disease [86, 87, 88, 89].

Therefore, neurodegenerative disease in the global population needs to be watched closely regarding long COVID patients as well as the long-term side effects of COVID-19 vaccination [90]. The increase of BBB permeability though tight junction alterations observed in inflammatory conditions like diabetes should be taken into account during vaccination [91]. Additionally, the spike protein of SARS-CoV-2 and the vaccine induces a decrease in serotonin by mobilizing ACE2, which aggravates or can cause depressive or even suicidal states [91, 93]. Of note, anosmia has been described following vaccination in COVID-19 negative subjects. This shows that symptoms identical to COVID-19 can appear and have been triggered only by spike protein [94]. It is likely that the chronic condition characterized by fatigue and neuropsychiatric symptoms, termed long-COVID and severe CNS damage could be induced by microglia activity and inflammation in astrocytes induced by spike protein from SARS-CoV-2 as well as from spike produced by vaccine [95].

Liver

Liver is also a target of COVID-19. Regarding vaccines, after 48 hours (with maximum concentrations observed between 8 to 48 hours), mRNA is found mainly in the liver (up to 21.5%), the adrenal glands, the spleen (≤ 1.1%) and the ovaries (≤ 0.1%). Thus, the liver is the organ where mRNA concentrates the most after COVID-19 vaccine injections. Furthermore, circulating spike protein can also accumulate in the portal vein after vaccination, as it is a key passage in the body regarding the central role of liver. The spike-induced mechanisms of inflammation described in this review can contribute to thrombosis in the portal vein. It has been proposed that among cirrhotic patients infected with COVID-19, portal vein thrombosis may be a potential complication even in the absence of hepatocellular carcinoma [96]. Portal vein thrombosis occurring after mRNA and DNA injections has been described in several studies [97, 98, 99]. In particular mechanical aspiration revealed massive acute thrombi from the extrahepatic portal vein and the superior and inferior mesenteric veins. Control venograms showed recanalisation of the portal venous system with minimal residual thrombi in the superior mesenteric vein [98]. The patient had received the first dose of ChAdOx1 nCov-19 vaccination (AstraZeneca) 11 days previously. These patients need to be followed carefully, as it can induce portal hypertension as well as oesophageal varices that can be deadly. Other reports describe hepatitis following vaccination, potentially involving autoimmune reactions, some of which are fatal [100, 101, 102].

Menstruations problems

Menstruations problems have been described in COVID-19. But they seem to be more frequent after vaccination. Lee KMN et al. reported that 42% of women with regular menstrual cycles bled more heavily than usual post vaccination [103]. Excessive and/or prolonged menstruation with or without irregular bleeding (metrorrhagia and menorrhagia), loss of periods (amenorrhea) and worsening of endometriosis condition have been observed. An analysis on VAERS data from 1990 until November 12, 2021 found 14,331 reports on menstrual disorders (1.36% of

total), of which 13,118 (90.90%) were exposed to COVID19 vaccine and 13,13 (9.10%) were exposed to another vaccine (ROR=7.83 for COVID-19 vaccines) [104]. Menstrual disorders were found increased particularly in the group of females aged 30–49 years, spiking to 97.4 % associated with COVID-19 vaccines among all other vaccines (vs 75 % for other ages), with a nearly tenfold increase in comparison with the pre-marketing period of the vaccine [104].

Various studies report changes after first dose between 25 % [105] and 50-60% of reproductive-age women [106] and up to 70 % after the 2nd dose [106]. Meanwhile these high values may be linked with some bias, for example when data were based on self-questionnaires from social media (LinkedIn, Facebook, and Twitter) among the general population, so women with menstrual issues could have been more motivated to answer than women who did not experience events [106]. The main sides effects were menorrhagia, metrorrhagia, and polymenorrhea (in 78,138 vaccinated females, 14 studies) [107] but also menstrual pain or cramps and abnormally heavy or prolonged bleeding [108, 109]. Also women with endometriosis seem more likely to experience changes in bleeding patterns (women with endometriosis: 39.5%, control group: 31.0%, p = 0.02) and a significant worsening in endometriosis-associated symptoms with an almost 4.3-fold worsening in dysmenorrhea [95% CI 1.9-9.9, p < 0.01] and 5.5-fold odds for any worsening in symptoms in endometriosis patients, as compared to the control group [95% CI 2.7-11.1, p < 0.01] [110]. In the majority of works, menstrual irregularities after both the first and second doses of the vaccine were found to self-resolve in approximately half the cases within two months. Meanwhile Zhang et al, in the VAERS study, also precise that only 18.01% of patients reported that their adverse reactions had vanished and 62.88% of patients reported that they still had adverse reactions related to vaccination [104].

Some authors suggest to consider these elements during the counselling of women who receive the COVID-19 vaccine which is mostly note done [106]. Regarding the mechanism of toxicity, menstrual cycle timing is regulated by the hypothalamic-pituitary-ovarian axis, which can be affected by life, environment, and health stressors. mRNA vaccines create a robust immune response or stressor, which could temporarily affect the hypothalamic-pituitary-ovarian axis [111]. The authors add that their results could not be explained by generalized pandemic stress because the unvaccinated control group showed no changes over a similar time period [111]. The spike protein receptor ACE2 is expressed in the ovaries, uterus, vagina, and placenta. Cytokines and glucocorticoids act on the hypothalamo-pituitary gonadal axis, arachidonic acid pathways, and the uterus, which leads to menstrual disturbances and pregnancy-related adverse events such as preterm labor and miscarriages [112]. Also, inflammation induced by spike protein from the virus or after

vaccine injection, could occur directly in the ovaries and uterus as evidence suggests that ACE2 is widely expressed in the ovary, uterus, vagina and placenta [113]. After 48 hours following injection (with maximum concentrations observed 8 to 48 hours), mRNA is found mainly in the liver (up to 21.5%), the adrenal glands, the spleen (≤ 1.1%) but also in the ovaries (≤ 0.1%).

Thus, we propose that the inflammatory pathways detailed in this review could also occur through circulating spike proteins at microvascular level in both the ovaries and uterus, leading to menstrual perturbations. We also hypothesize that it could provoke the induction of the luteal phase therefore explaining shorter menstruations until arrest of cycles in some cases. More studies should observe, even retrospectively, if possible, the evolution of hormones levels and inflammation in women after both severe COVID-19 and vaccination. Such inflammation in severe COVID-19 has been shown to induce preeclampsia conditions [114] and it could also occur with vaccine-induced inflammation with sur-activation of complement pathway for example [115]. Finally, even if some concerns have been raised regarding spontaneous abortions among vaccinated women in early pregnancy, no significant enhancement have been described so far in publications using VAERS or other data bases [116].

The issue of antibody-dependent enhancement (ADE) in with SARS-CoV-2 and after COVID-19 vaccination

ADE is a phenomenon in which suboptimal neutralizing or non-neutralizing cross-reactive antibodies bind to virus and facilitate Fcγ receptor mediated enhanced entry into host cells, followed by its replication, and thus increasing the cellular viral load. The production of facilitating antibodies has been observed in many viral diseases and after vaccination for dengue fever, Zika, Ebola, HIV, SARS-CoV, MERS-CoV, and feline infectious peritonitis [117, 118, 119, 120, 121, 122, 123]. In the current context, if the antibodies that are acquired by people who are vaccinated with the current vaccines, based on the mRNA of the initial SARS-CoV-2 (February 2020) become ineffective in destroying the delta variant or future variants, then these variants could produce facilitator antibodies. As a result, infected people may often have a more serious form of illness.

The modeling work published by Yahi et al. shows that antibodies facilitating the spread of the virus (ADE) have more affinity with the spike protein than neutralizing antibodies with regard to the delta variant (on the contrary to what is observed with the original strain of SARS-CoV-2 of 2020, Wuhan/D614G) [124]. This work highlights the need of evaluating the neutralization/ADE balance of the variants following the initial virus in the sera of vaccinated individuals, especially in the frame of a massive vaccination

campaign in order to adapt the vaccines to the most relevant and current variant [124]. The same team has also shown that a facilitating epitope located on the lower part of the N-terminal domain of the spike protein appeared to be highly conserved among most SARS-CoV-2 variants, which may represent a risk of antibody-dependent enhancement (ADE) [125]. Furthermore, ADE phenomenon occurs much more frequently with vaccines than with antibodies produced during an infection [126, 127]. Taken together, this could explain the observed ineffectiveness of COVID-19 vaccines against contamination and the very large number of vaccinated people who developed COVID-19 and died from it.

A few clinical studies have shown indications of ADE. According to Sridhar et al., ADE could be considered responsible for the aggravation of COVID-19 in the case of a woman recently vaccinated (7 days) with the mRNA BNT162b2 vaccine and who died from ARDS [128]. There was no trace of COVID-19 infection, however anti-spike antibodies were found on day 13 after injection. In another study, 2 patients with COVID-19 in the acute phase were vaccinated against COVID-19 with BNT162b2 vaccine (both on the 26th day of their COVID-19 illness) [129]. Both patients had COVID-19 pneumonia before receiving SARS-CoV-2 vaccination, and soon afterwards had new ground-glass opacities and respiratory failure. The authors suggest that the immune reaction to COVID-19 was reactivated by vaccination though they do not refer to ADE. Also, autopsies of 170 people who died from COVID-19 (or who were carriers of the virus at the time of death) showed lung viral loads much higher in the vaccinated group (n=29) than in the unvaccinated (n=141) (45% vs. 16%, respectively P = 0.008) and this was more significant in the partially vaccinated (n=16). The authors do not exclude the role of ADE in this phenomenon [130]. Finally, it also brings the discussion on the question of the safety of a large-scale vaccination during an epidemic or pandemic due to an RNA virus having a high propensity to mutate.

Specific inflammatory toxicity associated with mRNA vaccines

It is known that mRNA extracellular biomolecule can provoke inflammation, through toll-like receptors (TLR) and retinoic acid-inducible gene I (RIG-I) among other mechanisms, and predispose to endothelial damage, edema, hyper-coagulation and thromboembolic events, increases the permeability of the endothelial cells in brain [131] [132]. Furthermore before translation occurs, the mRNA may also bind to pattern recognition receptors (PRRs) within endosomes or cytosol and may also activate pro-inflammatory reactions such as inflammasome platforms, type I interferon (IFN) response and translocation of NF-κB [133].

Even if N1-methyl-pseudouridine presence in mRNA, should reduce its immunogenicity, this remain to be proven with more evidence [134] and very few mRNA vaccines have been tested on human subjects so far and only very recently [134]. In addition to mRNA and spike toxicity, of note is that the lipid nanoparticles (LNPs) are also known to cause clotting problems. This serious issue is present in a patent from Moderna on the injection of mRNA vaccine encapsulated in LNPs like in COVID-19 vaccines: "In some embodiments, the adverse effect includes coagulopathy, disseminated intravascular coagulation (DIC), vascular thrombosis, complement activation-related pseudo-allergy (CARPA), acute phase response (APR), or a combination thereof" [135].

This patent even provides for the addition of anticoagulants, antiallergics: "In some embodiments, the agent (molecule that can be added) inhibits platelet activation. In some embodiments, the agent is a platelet aggregation inhibitor. In some embodiments, the platelet aggregation inhibitor is aspirin or clopidrogrel (Plavix®). In certain embodiments, the platelet aggregation inhibitor is selected from aspirin/pravastatin, cilostazol, prasugrel, aspirin/dipyridamole, ticagrelor, cangrelor, elinogrel, dipyridamole and ticlopidine. In some embodiments, the agent inhibits CD36." [135]. Clearly, inflammatory effects of both mRNA and NLPs can be common and be additive with those of spike-induced inflammatory processes, including the complement pathway and may contribute to severe side effects as well as COVID-19 conditions if vaccination occurs during COVID-19 infection or long COVID conditions [29, 136].

Discussion

The spike protein of SARS-CoV-1 is 76-78% identical to that of SARS-CoV-2 [137]. Toxicity of spike protein has been documented for more than ten years with SARS-CoV-1 [138]. *In vivo* studies have demonstrated that spike protein of SARS-CoV-1 worsens acute lung failure through inflammatory pathways similar to SARS-CoV-2 [139, 140]. Also, studies dating back to SARS-CoV-1 and MERS-CoV outbreaks, have shown that vaccines based on the whole spike protein could induce a strong immune inflammatory response in many organs and particularly in the lungs and liver [141, 142]. In these *in vivo* studies in ferrets, not only did vaccination fail to prevent infection, but vaccinated animals showed significantly stronger inflammatory responses than control animals and focal necrosis in liver tissue. These studies and others have shown that vaccinate against coronaviruses is very challenging [143].

A study by Pfizer shows that radioactive mRNA was detected in most tissues from the first moments after injection (15 minutes) and the results confirm that the injection site and the liver are the main sites of distribution [84]. Low levels of radioactivity were detected in most tissues, with the highest levels in plasma being observed one to four hours

post dose. As previously referenced, at 48 hours mRNA is found mainly in the liver (up to 21.5%), the adrenal glands, the spleen ($\leq$ 1.1%) and the ovaries ($\leq$ 0.1%). It is imperative that additional research is done to evaluate precisely how long this toxic protein is produced and remains present in the cells of our organs but also in the bloodstream. It seems to be found after several weeks, with the possibility of producing chronic inflammation in many organs [3, 4, 5, 6, 83, 84]. The vaccines, which induce the production of spike protein, have a very strong inflammatory and oxidative potential over an undetermined period of time, given that the studies from the manufacturers have not yet provided this information. A study showed that the protein was found for at least 15 days after vaccination with Moderna, with a peak between one and five days at approximately 68 ng/L [3]. With the second injection (from 21 days) new spike proteins are produced, even as the antibodies and the body is working to eliminate this protein. Even if Ogata and his team observed a much shorter presence than for the first dose (a few days), there is potentially an inflammation which can be chronic and spread over several weeks, thus being able to destabilize the inflammatory balance in the blood vessels in the long term, the liver, brain, kidneys, etc. [3]. Röltgen K et al. have shown that mRNA vaccination stimulated robust germinal centers containing vaccine mRNA and spike antigen up to 8 weeks post vaccination in some cases [4]. Furthermore, the quantity of free spike protein in blood after COVID-19 vaccination has been found to be equivalent of that reached during an infection (up to 150 pg/ml) which could activate ACE2 receptors especially in organs where spike protein is more concentrated [144]. As a result, the vaccine may cause the same symptoms as COVID-19 but also potentially promote all inflammatory diseases in the medium and long term (cardiovascular, neurological, cancer, autoimmune).

This will pose a particular threat to those who already have an inflammatory disease (e.g. diabetes) or a family history of diabetes or inflammatory and autoimmune diseases [145]. Importantly, inflammation is always associated with oxidative stress in tissues. The crucial importance of oxidative stress in the pathology of COVID-19 has been largely underestimated and should be the subject of additional research efforts to identify antioxidant/anti-inflammatory treatments [146, 147]. Oxidative stress biomarkers predicted the severity and admission to intensive care of patients. Spike triggers inflammation and associated oxidative stress in COVID-19 through the biochemical pathways described in this article [148]. Moreover, it is known that oxidative stress, telomere shortening, cellular senescence, and body aging are associated [149, 150]. In COVID-19, telomere length in peripheral blood lymphocytes from patients aged 29 to 85 years has been found to be decreased in association with increased disease severity [151]. This could be linked with spike-induced aging as shown in study which measured the biological age of 117

COVID-19 survivors and 144 uninfected volunteers. The authors noted a significant increase in the biological age of patients who had COVID-19 of 10.45 years (+/- 7.29 years or 5.25 years beyond the norm) compared to 3.68 years (+/- 8.17 years) in uninfected people [152].

This phenomenon may occur both after COVID-19 infection when not treated, in long COVID, as well after vaccine in particular after various injections [153]. The toxicity of spike protein in vaccines needs to be studied and be better assessed. It is unclear if vaccines had a role to stop the pandemic and it cannot be ruled out that vaccine trials may have had a contribution in emerging variants of concern (VOC) (UK, Brazil, South Africa, India) escaping vaccine immunity and in emerging variants of interest [154]. A lot of scientists have asked for more information on vaccine safety, especially for children, given the large percentage of people who have been vaccinated. Also, it should be noted that preliminary studies on vaccines, such as AstraZeneca's for example, were on fairly young subjects and excluded participants with severe and/or uncontrolled cardiovascular, gastrointestinal, hepatic, renal, endocrine/metabolic, neurological diseases, as well as immunocompromised people and pregnant women [155]. This likely led to an underestimation of the serious side effects among the most vulnerable populations.

In conclusion, more research is needed on spike toxicity from SARS-CoV-2 but also by the spike protein produced by mRNA and DNA vaccines. Also more information is needed on the safety of LNPs, mRNA and adjuvants of COVID-19 vaccines which may contribute to the serious side effects observed after vaccination, including thrombosis. Lethality (case-fatality ratio) for COVID-19 is on average between 0.5 and 1% compared to 0.1% for the flu worldwide [156]. Urgent studies are needed to understand the benefits and risks of this vaccination, in particular among young people who are affected very minimally by the disease, and regarding the fact that vaccination did not prevent from contamination. This is still true for COVID-19 pandemic as well as for future mRNA vaccines, either mRNA vaccines for future pandemics, or mRNA vaccines for "classical" or "usual" vaccine preventable infectious diseases, especially those developed for children. These developments should be anticipated and discussed with more clarity, through a scientific and ethical dialogue. This could encourage many citizens, whether enlightened or not, to get vaccinated, otherwise there is an induced risk of a significant decrease in confidence in scientific and medical research and treatments [157].

Our attempt, in this review, was to highlight the main immuno-inflammatory pathways as well as the key role of oxidative stress in COVID-19. This is of central importance, especially after 3 years of pandemic, in order to propose better treatments that address precisely these immuno-

inflammatory pathways. We think that this has not been done yet and that it could lead to better results than what has been observed with dexamethasone for example. Furthermore, the toxicity of vaccine-induced spike protein has been neglected and underestimated since the beginning of the biggest mass vaccination in History, especially regarding what is already known on the high toxicity of spike protein. We have proposed various biochemical and physiological hypotheses in order to explain the severe side effects observed using mRNA and DNA injection technologies, often common with the severe forms of COVID-19 pathology and long COVID. This topic should be closely and carefully watched in the following years, especially on the incidence of inflammatory pathologies (cardiovascular, neurodegenerative diseases and cancers in particular) and aging.

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Conflicts of interest

All authors declare that they have no conflicts of interest.

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