SARS-COV-2 Vaccines Immunological Impact.

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Abstract. The immune responses to SARS-CoV-2 are herein detailed to clarify the innate immunity protective effects in a large fraction of individuals exposed to the infection, and the drawbacks of the interference of the acquired immunity cytotoxic T cells and antibody-dependent natural killer cell-mediated cytotoxicity arms. Very precisely, the available vaccines based on full-length spike glycoprotein in a mRNA or DNA-based construct, or whole virus potently lead to generation of these immunologically damaging effectors, especially following exposure to the pathogen. Conversely, a vaccine ~~exclusively~~ based on spike glycoprotein subunit 1 in a protein form can protect against the life-threatening virus infection and never lead to adverse side effects.

Keywords: SARS-CoV-2 vaccine; Innate immunity; Spike glycoprotein subunit 1; Viral peptides presentation; HLA class I and class II; Neutralizing antibodies; Cytotoxic T lymphocytes; Natural killer cells; Inflammasome activation; Cytokine storm

1. Introduction

The dramatic world-wide transmission of the SARS-CoV-2 has triggered disease, fear, uncertainty, and an explosion of often obscure, confusing, and controversial information directed to the scientific community and the public. Since the start of the COVID-19 outbreak, the rampant and unprecedented race for developing vaccines based on the whole virus, or full-length spike glycoprotein, which is instrumental in mediating invasion of human structural cells, has led to emergency authorization of various vaccines. The lack of thorough testing in animals prior to clinical trials, authorizations based on safety data generated during trials that lasted less than 3 months, and the high rate of occurrence of a wide range of adverse effects, raised questions regarding the safety of these vaccines. Accordingly, it was necessary to provide details for a better understanding of the immunological risks presented by the corona virus and by the available vaccines administered to the SARS-CoV-2-naive and previously exposed individuals. Additionally, we show for the first time that the risks of SARS-CoV-2 infection and vaccination, serious sequelae in a substantial fraction of the world population, and global erosion of public confidence in science and health committees and organizations could be avoided via developing a vaccine based on use of the spike glycoprotein subunit 1 in a protein formulation.

2. Data

2.1. The pathogen

Severe acute respiratory syndrome (SARS) corona virus (CoV) is termed SARS-CoV-2 because it is similar to a SARS enveloped, positive sense, single stranded RNA CoV, which caused an outbreak predominantly in Asia in 2003 and found to infect humans and bats lung epithelial cells via binding to the cell surface transmembrane carboxypeptidase, angiotensin-converting enzyme 2 (ACE-2). The causative virus was named SARS-CoV, and its 29,881-base genome was sequenced in April 2003 [1]. SARS-CoV-2 induced a pandemic disease outbreak in 2019 and was thus designated COVID-19. SARS-CoV-2

appeared to transmit from human-to-human more rapidly than SARS-CoV [2,3]. The SARS-CoV-2 RNA genome (29,903 nucleotides) has been sequenced, revealing around 90% overall nucleotide similarity to a group of SARS-like coronaviruses (family *Coronaviridae*, genus *Betacoronavirus*, subgenus *Sarbecovirus*) that had previously been found in bats, and not known to readily infect humans. The receptor binding domain (RBD) located in the spike protein is, however, more closely related to that of SARS-CoV, and only one amino acid longer [4-6].

SARS CoV-2 has a total of 11 genes with 11 open reading frames (ORFs): *ORF1ab, ORF2, ORF3a, ORF4, ORF5, ORF6, ORF7a, ORF7b, ORF8, ORF9*, and *ORF10*. The first gene (5' to 3') *ORF1ab*, encodes a large polyprotein that proteolytically cleaves to form 16 NON-STRUCTURAL proteins (NSP). The STRUCTURAL spike (1273 amino acids, aa), envelope (75 aa), membrane (222 aa), and nucleocapsid (419 aa) proteins are encoded by *ORF2, ORF4, ORF5 and ORF9*, respectively. *Open reading frames 3a, 7a, 7b, 8, and 10* encode ACCESSORY proteins of 38 to 275 aa, reported to be of limited importance for virus replication in vitro, yet likely critical for in vivo virus-host interactions [7,8]. The single-stranded RNA genome is packaged within a capsid, which consists of the nucleocapsid protein (N) surrounded by a membrane that contains the membrane (M) and the envelope (E) proteins, involved in virus budding, and the spike glycoprotein (S), where the RBD is located [5-8] **(Fig. 1**).

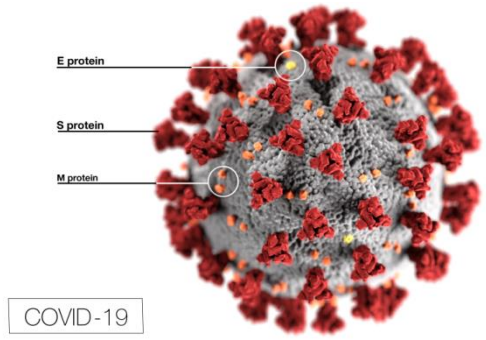

Figure 1. SARS-CoV-2 cartoon. From Centers for Disease Control and Prevention (CDC); credit: Alissa Eckert, MSMI, Dan Higgins, MAMS.

2.2. Uptake by innate immune cells

2.2.1. First encounter

SARS-CoV-2-containing droplets may be inhaled and land in the nose and on the mucosa lining the buccal cavity. Cilia and mucus in the nose cavity and upper trachea are effective in virus entrapment and removal. Soluble mucins' glycoproteins are charged with terminal sialic acid and gangliosides [9,10]. SARS-CoV-2 readily binds to sialic acid- and gangliosides-containing glycoproteins via a sialic acid-binding domain at the amino terminal domain (NTD) of the S glycoprotein, and may, thus, be readily removed by the mucociliatory escalator (**Fig. 2A**) [9-12]. However, caution and stringent hygiene must be exercised during disposal as the virus remains stable in mucus, saliva, and sputum for up to 48 h, especially at low temperature and humidity, and is, thus, potentially contagious for other persons [13].

2.2.2. The complement system stands up

Entrapped and free virions are targeted by natural antibodies, lectins, or complement 3 in nasal secretions and saliva [14-16], resulting in activation of the complement cascade, lysis of the virions, and generation of chemotactic and opsonic complement products in the nasopharynx (**Fig. 2B**) [17]. Complement (C) anaphylatoxins, C3a, C4a, and C5a, bind to specific receptors on the surface membrane of mast cells in the nasopharynx [17].

Additionally, SARS-CoV-2 may directly activate resident mast cells via interaction with their surface membrane innate immunity receptors, namely toll-like receptors (TLR). Mast cells activation elicits release of histamine, proteases, cytokines, chemokines and arachidonic acid compounds, such as prostaglandin D2 and leukotrienes, involved in the impairment of local blood capillary endothelium integrity with subsequent edema and neutrophils extravasation (**Fig. 2C**) [18-20]. Complement 3b and C5b molecules cover the virus particles, promoting their phagocytosis by resident macrophages and dendritic cells and recruited neutrophils (**Fig. 2D**) [17]. Virus particles may also be endocytosed following binding the different pattern recognition receptors (PRR) expressed on the surface of neutrophils, macrophages, dendritic, and epithelial cells. Lysosomal enzymes mediate destruction of the virus, and release of interleukin (IL)-1 follows [21-23]. These changes explain the fever, headache, throat soreness, inflammatory congestion in nose and mouth, and cough; all, however, are indications of serious defense mechanisms of the innate immune system [23-26].

2.2.3. The type I and III interferon saga

Virus particles in the endosomes of macrophages and dendritic cells uncoat, releasing their single-stranded RNA (**Fig. 2E**), which is immediately bound by TLR 7, with consequent release of inflammatory cytokines and the viricidal interferons (IFN) alpha, beta (type I), and lambda (type III) [26]. Type I and type III IFN are a most potent defense system against virus replication and spread. Of note, SARS-CoV-2 NSP lead to reduction in the amount and function of these highly protective molecules, especially in adults [23,26]. Leakage of viral RNA from the immune cells endosomes to the cytoplasm engages the cytoplasmic pattern recognition receptors (PRR), notably retinoic acid inducible gene 1 (RIG-1) (**Fig. 2F**). Binding of viral RNA to RIG-1 helicase domain initiates a signal transduction cascade pathway with subsequent generation of inflammatory cytokines and IFN type I and III [26,27], and activation of the inflammasome, which mediates the exit of IL-1 and IL-18 from the cell to the surrounding milieu. The related receptor, melanoma differentiation-associated protein 5 (MDA5) appears to play a marginal role in coronaviridae infection [28]. In support, MDA5 was shown to be a major sensor of SARS-CoV-2 in human lung epithelial cells leading to production of type I IFN, which was, however, unable to control viral replication [29]. Individual differences in host/virus control of type I IFN production and function are instrumental in dictating an almost asymptomatic, mild, or severe COVID-19 course [26,30]. SARS-CoV-2 uses a multi-arm strategy to antagonize the IFN response, with low levels often reported in several studies [26,30-35]. SARS-CoV-2 NSP1, a potent inhibitor of host gene expression; NSP8, a component of the virus RNA polymerase; NSP13, the virus helicase which unwinds duplex RNA; and NSP16, responsible for synthesis of the viral RNA 5' cap readily antagonize IFN signaling, cause decrease in type I IFN production by infected cells (**Fig. 2F**), and reduce expression of IFN-stimulated genes (ISG) [7,26,31,36-38]. The SARS-CoV-2 proteins encoded by the *membrane (M)* gene and *ORF9b*, an alternative open reading frame within the *nucleocapsid (N)* gene, were found to target mitochondrial membrane and impair RIG-I/MDA-5 signaling, antagonizing type I IFN production, the major arm in inhibition of viral replication and innate antiviral immunity [34,35,39]. The MDA-5 poor anti-SARS-CoV-2 innate immunity response [28,29] was recently shown to result from the virus papain-like protease (NSP3) inhibition of the receptor oligomerization, a necessary step for initiation of the signal transduction cascade [7,40]. SARS-CoV-2 eradication and infection overcoming will certainly not take place if SARS-CoV-2 strain and load, host underlying health conditions, pre-existing comorbidities, age, gender, inborn genetic flaws, autoantibodies to IFN, medications and drugs intake, and other still undefined factors specific to every individual lead to aberrant type I IFN production and deregulated antiviral activity, perhaps associated with normal or exacerbated release of inflammatory cytokines [26-45].

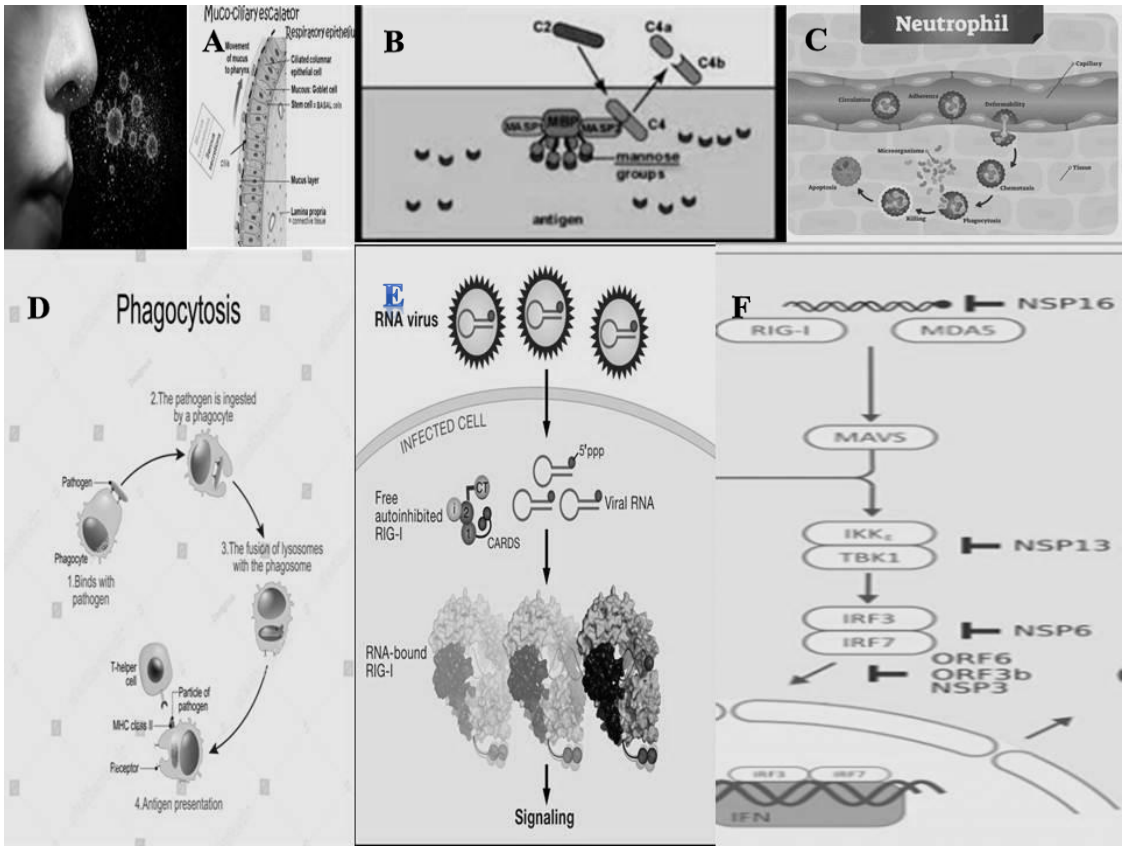

Figure 2. Inhaled SARS-CoV-2 are met by the innate immune defenses, notably the mucociliatory escalator (**A**) and activated complement system (**B**) that predominantly facilitates neutrophil and other immune cells extravasation (**C**) and virus phagocytosis (**D**). In the immune cells endosomes and cytosols, the virus RNA binding to the innate immunity receptors activates signaling pathways that elicit production of inflammatory cytokines and type I interferon (**E**). Several virus nonstructural proteins (NSP) antagonize receptor activation and signaling (**F**).

2.2.4. Virus proteins fate in the sentinel cells

SARS-CoV-2 NSP, structural, and accessory proteins released in immune cells' endosomes and cytoplasm undergo proteolytic digestion by the endosomal and proteasome proteases, respectively, followed by presentation of the viral peptides in association with HLA class II and class I, respectively on the surface of the immune cell (**Fig. 3A**). The cytokines produced in response to the viral RNA interaction with innate immune receptors enhance the viral peptides antigen (Ag) presentation. Fully activated Ag presenting cells (APC) migrate to lymph nodes for activation of naïve T cells and B cells. It is important to note that the human immune system sentinel cells, dendritic cells, monocytes, and macrophages do not support virus replication [26,46-48], and are not responsible for virus spread. They are focused on Ag presentation and production of co-stimulatory molecules and cytokines to elicit the generation of T cytotoxic and T helper lymphocytes, and activated B cells-derived antibodies specific to the various virus peptides, within a week or ten days of virus uptake [49]. By this time, however, the innate immune defenses of many, but not all, individuals have succeeded in clearing the virus from the nasopharynx. The cytotoxic T cells have only to target and kill the few infected structural cells, as well as, the APC with viral peptides/HLA class I complexes on their surface (**Fig. 3B**), thus, dampening the host acquired immune responses [48]. Resolution of the inflammation and generation of memory T and B cells follow [26,50].

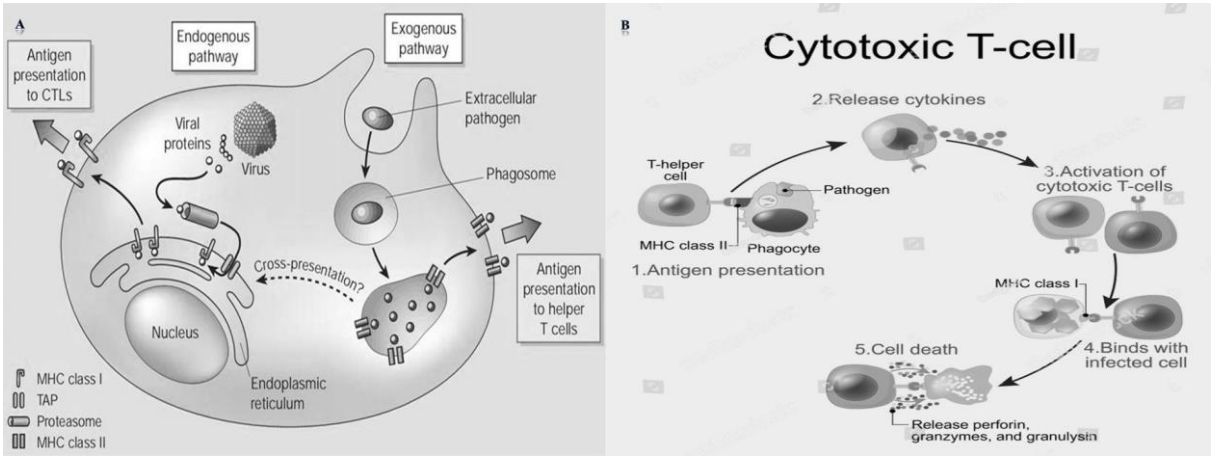

Figure 3. Released SARS-CoV-2 proteins are processed for surface membrane antigen presentation in association with MHC class I and class II molecules **(A)**, ready to activate the acquired immune system. Immunologic risks are due to the cytotoxic T cells which are poised to kill any structural cell presenting the specific viral peptide/MHC class I complex on their surface membrane **(B)**. .

2.3. Invasion of structural cells

2.3.1. A multi-step infestation

A load of SARS-CoV-2 can evade uptake by immune cells in the nasopharynx and infect a high number of different tissues provided their cells possess on their surface membrane the virus specific receptor, ACE-2, recognized and bound by the virus RBD, and S protein-activating proteases, namely furin and transmembrane serine protease 2 (TMPRSS2). The S protein is homotrimeric as is clearly illustrated in Figure 1. The primary structure of the spike protein monomer reveals an ectodomain, a trans viral membrane protein, and an intra virion cytoplasmic tail. At the amino end of the ectodomain, the signal sequence is followed by the NTD, then the RBD of 223 aa, containing viral key aa residues recognized by the extracellular peptidase domain of ACE-2, thus allowing virus entry into host structural cells [4-6].

Following binding of RBD to the cell surface membrane ACE-2, the spike protein is cleaved at the S1/S2 fully exposed protease cleavage site into the RBD-containing S1 subunit at the amino end and the S2 subunit at the carboxyl end. This cleavage site presents multiple basic residues (Arg-Arg-Ala-Arg sequence, aa 682–685) that form an exposed loop, allowing the specific sequence cleavage by several proteases, including furin. Upon fusion with the ACE-2 receptor, the receptor-binding subunit 1 (S1) is cleaved and shed. Despite that the virus interacts with ACE-2 via a single S1 subunit, disassociation of one S1-ACE-2 from the S trimer could cause sequential disassociation of the S1 subunits from the spike trimer. Upon the start of virus infection of structural cells, the three S1 subunits from the spike dissociate from the virion and are shed outside of the cell [51,52].

The S2 subunit is responsible for virus/cell membrane fusion, and contains a second protease cleavage site, S2', which is completely buried in the prefusion spike glycoprotein, a hydrophobic fusion peptide, and two heptad (seven amino acids) repeats, located adjacent to hydrophobic, potentially fusion-related regions in the aa sequences of coronaviruses**.** The fusion subunit (S2) undergoes large-scale conformational rearrangements to expose the hydrophobic fusion peptide, and brings the viral and cellular membranes close for fusion. The coronavirus spike protein binding to the cell membrane is further activated by specific cell enzymes. Genomic analyses of the new coronavirus have revealed that its spike protein differs from those of close relatives. A major difference lies in SARS-CoV-2 spike protein displays a second protease cleavage site (S2', FP) that is activated by a host-cell surface membrane enzyme, TMPRSS2 found in the lung, liver,

kidney, and small intestines. Cleavage of the S2′ site by host cell proteases is required for successful infection by SARS-CoV [1,4-6,26,49,51,52].

2.3.2. Susceptible cells

Human cells which express surface membrane ACE-2 and TMPRSS2 are susceptible for SARS-CoV-2 invasion. Single cell RNA sequencing revealed that the lung, heart, esophagus, ileum, kidney, bladder, and nervous system are at risk of the infection, with type II lung alveolar, heart myocardial, ileum and esophagus epithelial, kidney proximal tubule, and bladder urothelial cells showing highest ACE-2 expression. The stomach and the liver showed no cells expressing ACE-2 [53]. Single cell gene expression of ACE2 and S protein-activating proteases confirmed that ACE-2 and virus invasion cofactors were mainly expressed in lung type II alveolar cells, esophagus keratinocytes, colon colonocytes, ileum and rectum epithelia, and kidney proximal tubule, but contrary to the previous study, expression was also evident in stomach epithelial cells and liver cholangiocytes [54]. The whole digestive system is hence a major target of infection, but does not represent an immense threat as its cells are continuously dividing and renewing. Single cell RNA expression of genes encoding ACE-2 and TMPRSS2 revealed that cells, which express concomitantly high levels of both genes, are restricted to the nasal cavity ciliated and secretory cells, lung bronchi secretory and parenchyma alveolar type II, cornea superficial conjunctiva, ileum and colon enterocytes and progenitor cells, common bile ducts and gallbladder [54]. Fortunately, fetal tissues had cells expressing both genes only in the medullary thymic epithelial cells [54], however, central in T cell education and maturation [50]. Additionally, human blastocyte-stage pre-implantation embryos robustly expressed the canonical SARS-CoV-2 entry receptor *ACE-2* and the putative activator protease *TMPRSS2*, in addition to other reported entry factors [55].

The critical point for SARS-CoV-2 entry of host cells is their expression of ACE-2, as TMPRSS2 may be replaced by the activity of several other proteases. Single cell RNA sequencing and immunohistochemistry showed that the cells of the cornea display high ACE-2 expression [54,56]. Single cell RNA sequencing, Western blot, confocal microscopy and immunohistochemistry assays confirmed the expression of the SARS-CoV-2 receptors ACE-2 and TMPRSS2 in human conjunctival epithelial cell line and in the human conjunctival, limbal and corneal epithelium [56-59]. These data indicate that the eye is a potential port of entry and route for the transmission of SARS-CoV-2, while there is no firm recommendation for wearing eye glasses or shields. High expression of ACE-2 was additionally detected in the stromal cells of the heart, liver cholangiocytes, kidney proximal tubules, and testis myoid and spermatogonial stem cells [54]. ACE-2 expression was not detected in the skin, skeletal muscle, brain, and spleen [52-54]. ACE-2 protein expression was, however, reported in the mouse and human brain, notably in hypothalamic regions associated with food intake and metabolic regulation. Additionally, ACE-2 expression was evident using immunohistochemical assays in the endothelial cells of veins and arteries, and artery smooth muscle cells in oral and nasal mucosa, nasopharynx, lung, stomach, small intestine, colon, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney, and brain [60-63]. The susceptibility of endothelial cells to SARS-CoV-2 infection was recently challenged [64]. Of note, the pancreas exocrine and endocrine cells, thyroid and testes are also susceptible to SARS-CoV-2 invasion as they express ACE-2 and TMPRSS2 in proximity [65]. Recent report documenting the expression of SARS-CoV-2 entry molecules on the surface membrane of human hematopoietic stem cells and endothelial progenitor cells raises concerns the virus infection may damage the stem cell compartment [66].

2.3.3. A bleak scenario

Upon SARS-CoV-2 invasion of target cells, the viral and endosomal membranes fuse, releasing the viral RNA into the cytoplasm [67]. The viral RNA interacts with the cytoplasmic innate immunity receptor, RIG-1, attempting to elicit production of the viricidal interferon alpha and beta, and inflammatory cytokines, namely IL-1, responsible for fever. If the human host is, additionally, able to generate antibodies that hinder and prevent virus entry into structural cells, he/or she will still resist the infection, presenting only light or moderate symptoms [68].

Upon massive virus entry into the target lung, kidney, and small intestine cells, proteolytic processing of the intracellularly released viral proteins will take place. Of note, the outcome of viral proteins proteolysis and processing differs with cells types and individuals, subject to protease activity restrictions. Innumerable viral peptides in association with HLA class I molecules will be presented on the surface membrane of the virus-infected cell, poised to stimulate pre-cytotoxic CD8+ lymphocytes [50]. Viral peptides presentation is subject to the laws of HLA restrictions in Ag presentation [26,50]. Virus robustly replicates in the cytosol, eventually leading to infected cell death by pyroptosis [26,49,66]. Concurrently, macrophages and dendritic cells that have captured the virus process and present peptides/HLA class I and class II molecules on their surface membrane ready to activate the helper CD4+ lymphocytes, which help in the proliferation and differentiation of killer CD8+ and antibody producing B cells [26,50,67-73]. The cytotoxic T cells will target the virally infected structural cells, are able to target them all, and lead to excessive damage of key organs such as the lung, digestive system, heart, brain, and kidney. Antibodies may bind to viral peptides on the surface of the host cells and engage natural killer cells to devastating antibody-dependent cell-mediated cytotoxicity (ADCC). Virus- and immune responses-mediated cell injury and death release an immense plethora of danger-associated molecular patterns (DAMP), responsible for firing the cytokine storm and widespread inflammation, which may well lead to host death [26,49]. It is best that such immune responses are never generated.

2.4. COVID-19 vaccines in world-wide trials

2.4.1. Pfizer-BioNtech and Moderna vaccines

Pfizer BNT162b2 and Moderna mRNA-1273 are lipid nanoparticle-formulated, nucleoside-modified RNA vaccine that encodes membrane-anchored SARS-CoV-2 full-length spike protein, modified by two proline mutations to lock it in the prefusion conformation [74]. The vaccines are to be given intramuscularly (IM). The lipid nanoparticles assist the entry of the mRNA in the cytosol of cells, the realm of which is as yet incompletely defined. Particles may escape in blood capillaries, disseminate, and deliver their cargo in endothelial cells and structural cells anywhere in the vaccinee organs especially that the pharmaceutical laboratories have not yet presented data on the mRNA location, persistence, or translation product(s) in the body. Hopefully, a part or the entire bolus is endocytosed by macrophages, monocytes, dendritic cells or B cells. That would be the best fate for such type of injection, but the IM immunization fails to exactly target these cells. The foreign mRNA uses host cells ribosomes to synthesize the SARS-CoV-2 spike proteins that may leak to the surrounding fluids to be endocytosed by immune cells for potential Ag presentation, and activation of the acquired humoral and cellular immune responses [50]. The cytoplasmic proteasome or immune proteasome will never fail to proteolytically digest some of the foreign spike subunit 1 and 2 proteins, followed by their peptides presentation on the cell surface membrane in association with HLA class I molecules. The processes are evidently subject to the proteolytic enzymes- and HLA- restrictions in Ag presentation [50]. Two 30-μg doses of BNT162b2 or mRNA-1273 elicited high SARS-CoV-2 neutralizing antibody titers and robust antigen-specific CD8+ and Th1-type CD4+ T-cell responses [67,74-77].

2.4.2. Oxford/AstraZeneca simian adenovirus-vectored vaccine

The Oxford University/AstraZeneca's simian adenovirus-vectored AZD1222 vaccine is based on the chimpanzee adenovirus-vectored platform (ChAdOx1/AZD1222) that has had its original DNA genetic removed and substituted with DNA, encoding a codon-optimized, full-length spike glycoprotein of SARS-CoV-2. These adenovirus particles do not carry the viral machinery needed for replication. They do have all the viral machinery needed to infect human cells and force them to express the coronavirus spike protein. The original viral leader sequence has been replaced with the leader sequence of the human tissue plasminogen activase protein, which enhances immunogenicity and immune responses. The vaccine is administered IM, and the map of cells targeted is not defined. The adenovirus is translocated into the nucleus, with landing spot unknown, but where the gene for the coronavirus spike protein can be read by the cell and copied into mRNA. Any cell in the host tissue and organs may be invaded, harbor the foreign gene(s) and express the spike protein. Where the adenovirus and its cargo intercalates and integrates? Near which gene, for how long, for months or perhaps years? DNA vaccine apparently shows negligible levels of integration into host cellular DNA; that unlikely event may, however, result in chromosomal instability, and worse, cause insertional mutagenesis leading to the activation of oncogenes [78]. Pharmaceutical laboratories should have by now presented data on the location and expressed products of the DNA injected in the body. Structural cells will present the spike protein peptides on their surface in association with MHC class I molecules. It is a must that the vaccine accesses APC for triggering immune responses. DNA is far more stable than mRNA, and it is not clear how long the virus construct will persist in the cell nucleus or cytosol. Product mRNA-mediated triggering of the innate immunity receptors such as TLR3, TLR7, RIG-1, MDA-5, and the inflammasome, and production of type I IFN and inflammatory cytokines continue until the foreign DNA is degraded. A single dose of the vaccine was shown to induce Th1-biased cellular and antibody immune responses [78-83].

2.4.3. The single dose Johnson & Johnson/Janssen vaccine

The Janssen AdVac® viral vector technology is based on a non-replicating, genetically manipulated adenovirus serotype 26 virus that has been genetically modified to contain the gene encoding the full-length spike protein of the SARS-CoV-2 virus in a stabilized conformation. The vaccine, JNJ-78436735 or J&J; Ad26.COV2.S can remain stable for months at 4°C perhaps because it additionally contains citric acid monohydrate, trisodium citrate dihydrate, ethanol, 2-hydroxypropyl-β-cyclodextrin, polysorbate 80, sodium chloride, sodium hydroxide, and hydrochloric acid. The vaccine can induce potent and long-lasting binding and neutralizing antibody and type 1 helper and cytotoxic T cell immune responses [84-86], despite that the producing company did not present as yet information on the location or duration of the viral vector persistence in the host cells.

2.4.4. Sputnik V COVID-19 vaccines

The two vaccines are based on genetically modified adenovirus-type 26 or type 5 vectors, which carry the gene for SARS-CoV-2 full-length glycoprotein S (rAd26-S and rAd5-S). Both components were developed, manufactured, and stored by N F Gamaleya National Research Centre for Epidemiology and Microbiology (Moscow, Russia). The vaccines were administered IM alone (one dose), or with rAd26-S given on day 0 and rAd5-S on day 21. The heterologous rAd26 and rAd5 vector-based COVID-19 vaccine have a good safety profile and induced strong humoral and cellular immune responses in participants. Vaccine efficacy reached 91.6% [87,88]. Further information are not readily available.

2.4.5. The virus-attenuated Sinovac's Corona Vac (formerly PicoVac)

SARS-CoV-2 is retrieved from cultured Vero cells, inactivated with *B*-propiolactone, and purified. Alum is used as adjuvant, and the vaccine administered IM. The whole virus vaccine's immunogenicity, safety and immune protection were confirmed in mice, rats, guinea pigs, rabbits, rhesus monkeys, and volunteer humans [89]. The vaccine is certainly safe regarding entire absence of induction of viral RNA or DNA-mediated intercalation, integration, or other modification processes in the genetic machinery of the vaccinee cells [89-91].

2.5. Vaccine-mediated immunological outcomes

2.5.1. In SARS-CoV-2-naïve individuals

Constructs containing inactivated virus or spike protein are endocytosed or phagocytosed by neutrophils. macrophages, dendritic cells, and B lymphocytes, processed for viral Ag presentation and induction of the generation of T helper 1, T helper 2, T helper 17, and cytotoxic T cells capable of recognizing a plethora of cell surface membrane viral peptides in association with HLA molecules class I and II {50,76]. Activated B lymphocytes produce antibodies capable of recognizing numerous linear and conformational epitopes in viral molecules. Nothing otherwise happens, even after the boost immunization, except for generation of memory T and B cells ready for action if similar pathogen determinants are seen by the immune effectors [89-91]. In support, it was predicted that the inactivated Covid-19 vaccine will make a global impact [92], and the Novavax vaccine, spike 1 and 2 subunits in a protein construct,is highly effective, safe, and practical [93].

More serious events might theoretically occur with mRNA encoding S protein subunit 1 and 2 accessing the cytosol of dividing or non-dividing cells, anywhere in the vaccinee tissues and organs. The vaccine within the endosomes and cytosol of APC triggers generation of acquired immunity directed to the peptides of the virus S protein. There is no virus for antibodies to neutralize, agglutinate, or opsonize, but cytotoxic T lymphocytes and antibodies will never fail to find the cells with the vaccine mRNA-induced viral peptides on their surface. Direct cytotoxic T cell killing and antibody-dependent natural killer cell cytotoxicity (ADCC) will eliminate the target cells, irrespective of their importance for the body. Serious inflammation follows, but usually subsides [76-78; 94-98]. The only serious outcome is potential irreversible loss of perhaps key and indispensable organ cells, differently from other respiratory virus vaccines, such as split-virus, inactivated influenza A (H1N1) vaccine H1N1, which predominantly access APC and elicit the production of immune effectors, which have to wait for any virus infection of the respiratory tree cells and expression of foreign or cross-reacting peptides on their surface membrane [99,100]. On the contrary, mRNA vaccine- loaded cells express all the peptides of spike subunit 1 and 2 and are the immediate targets of immune cell killing, irrespectively and independently of any virus infection. The reactions are transient and may result in no serious or persistent damage except in case the cells are of critical importance and limited regenerative ability.

Outcome of vaccination with the adenovirus-harbored DNA genes depends on whether the DNA in the nucleus is or is not transcribed into mRNA, which translates in the cytosol to S protein subunit 1 and 2. The cells which have integrated the foreign DNA in their genome or unable to transcribe it shall not be recognized by the generated immune effectors, and may survive or continue division with uncertain and unpredictable course. Expression of the viral peptides in APC and other cells will activate the acquired immunity system with eventual elimination of all immune and structural cells harboring the vaccine in expression mode. Inflammation scenario, scope, and consequences are related to the site of immune attack, and may well vary from allergic reaction, thrombocytopenia

and thrombosis, to strokes, and endocrine and neurological disorders [101,102]. The central flaw of the nucleic acid vaccines is the failure to direct or predict their landing point.

2.5.2. In SARS-CoV-2-previously exposed individuals

It is now documented that immunity in convalescent individuals will be very long lasting [72,103,104]. Immunological responses to the inactivated virus vaccine or immunogens S protein subunit 1 and 2 in a mRNA or DNA construct proceed in SARS-CoV-2 pre-exposed as in naïve individuals, albeit in a faster rate and with greater amplitude. Neutralizing and opsonic antibodies are slowly catabolized [77]. The vaccine immunological impact is more serious than in naïve individuals, in the event that besides the mRNA and DNA vaccine, previous SARC-CoV-2 virus exposure has resulted in eliciting susceptibility to cytotoxic T cell killing and ADCC in large populations or subpopulations of cells in particularly vulnerable organs. All these cells are destined to die, with generation of a plethora of danger-associated molecular patterns (DAMP), cytokine storm, and overt inflammation [105]. Except for the unlikely generation of genetic changes in key cells [106], the previous infection and the vaccine will end by arming the host with an array of memory T and B cells ready for fighting the same or similar invader strain. Vaccine protection is justified and required against infection with what indeed should be termed multi-organ dysfunction syndrome-corona virus 2 (MODS-C0V-2), as recently recommended [107].

2.5.3. SARS-CoV-2 infection prognosis in vaccinees

Virus infection proceeds as detailed in sections 3 and 4, with virus processed in immune cells, and virus invasion, replication, and Ag presentation in structural cells of the nasopharynx and upper respiratory tree. Within 3 days, the memory immune responses are fired up. Antibodies specific to every virus protein in killed virus- and to S protein subunit 1 and 2 in nucleic acid-vaccinees will join the natural antibodies and plasma lectins in activating all pathways of complement activation; will induce rapid and efficient virus agglutination, and opsonization into resident APC; while antibodies specific to the RBD of S protein subunit 1 will prevent the pathogen from invasion of further structural cells. Variants showing differences from the strain used for vaccination may not be susceptible to antibody-dependent neutralization, but still remain targets for antibody-mediated agglutination and opsonization. The problem involved the structural cells where the virus has succeeded to invade. The cytotoxic T cells to numerous viral peptides and to S protein subunit 2 only which the inactivated and nucleic acid vaccines have generated, respectively will eliminate every such cell, wherever they are at the cost of the perfect balance in the physiology, biochemistry, and function of impaired cells, tissues, and organs. Cell death, spread virus, and DAMP elicit inflammation, cytokine storm, and severe disease. The cytotoxic T lymphocytes activities (which may be supported by ADCC) proceed in parallel with the neutralizing (if no mutations occurred in the RBD) and opsonizing services of the antibodies, and the disease, even severe, will almost certainly never go as far as death of the host. The host will survive but with one or more of an entirely individual-specific, random and unpredictable choice of sequelae, ranging from strokes, diabetes, endocrine dysfunctions to early Alzheimer, parkinsonism, and cancer [108-112].

2.6. The safe spike subunit 1 PROTEIN vaccine

In naïve or SARS-CoV-2 pre-exposed persons, spike glycoprotein subunit 1 or RBD **protein** immunogen will be captured by APC leading to generation of specific antibodies, and T helper and cytotoxic antibodies. Yet, the killer cells and the antibodies specific to the S1 subunit will not approach the SARS-CoV-2-infected cells, and the host critical organs would remain spared from potentially deleterious immune reactions, because spike

glycoprotein subunit 1-derived peptides are likely not or poorly presented on the cells' surface membrane. Upon viral challenge infection, antibodies to the subunit 1 will neutralize the virus preventing entry into structural cells or lead to its agglutination and opsonization, while the memory adaptive response is activated. Specific cytotoxic T lymphocytes or natural killer cell-activating antibodies will never approach virus-infected structural cells, as they do not present the spike glycoprotein subunit 1 on their surface. The safety of such vaccine has been demonstrated [113-115]. It is relieving that an efficacious and safe RBD-based vaccine in a recombinant protein construct is now in clinical testing [116-118]. The S1 subunit is composed of 671 aa, expected to elicit the generation of a plethora of various and diverse antibodies and long-lived plasma cells, effective against virus variants. In the event of considerable mutations in the viral RBD impairing antibody neutralization roles, the antibodies may still counteract infection and limit virus spread via enhancing virus agglutination, opsonization, and complement-mediated attrition. Conversely, few circulating products of the mRNA vaccine have recently been detected only after the first, but not after the second, dose [119].

Administration of the spike glycoprotein subunit 1 or RBD immunogen in the form of mRNA [120]- or DNA-based vaccine will allow random and unpredictable entry of the mRNA and DNA construct into cells, which could be of critical importance for the host welfare. It obliges host cells to internalize S1 and present its peptides on their surface membrane. These cells will be the target of killer cells even during the first and especially the boost vaccination, depending on the probability and randomness of S1 cells import and their proteolytic and antigen presentation processes and machinery. There will be no risk, however, upon virus infection.

The danger of use of the full-length spike glycoprotein or the whole virus in a protein, RNA, or DNA construct is detailed above, but is justified by the extreme severity of the virus infection [107], especially the B cells, monocytes, eosinophils, basophils, and cytotoxic cells such as CD8, γδ T cells, and natural killer cells lymphopenia [121,122], which could be ascribed to extravasation to the sites of viral injury, or to viral invasion and pyroptosis of the hematopoietic stem/progenitor cells that all express functional ACE-2 [66,123].

3. Conclusions

Upon viral infection, viral peptides are processed in the cell endosomes and cytosol for eventual presentation on the surface membrane in association with HLA molecules. Virus-infected structural cells presenting numerous viral peptides on their surface membrane are targets for cytotoxic T cells and for antibody-dependent natural killer cell-mediated cytotoxicity. Since the SARS-CoV-2 spike glycoprotein subunit 1 is the only viral polypeptide, which does not penetrate host structural cells, it is not presented on their surface membrane in association with HLA class I molecules. Accordingly, a safe SARS-CoV-2 safe and efficacious vaccine should be based on spike glycoprotein subunit 1 in protein form. Such vaccine will principally induce protective antibodies, which interfere with the processes of viral invasion of critical host cells in the heart, lung, liver, kidney, and small intestine, and promote phagocytosis and elimination of virus particles. This vaccine fails to induce generation of cytotoxic T cells that threaten virus-infected structural cells, hence sparing organs, especially the lung and brain, from severe damage, often leading to substantial morbidity and mortality.

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Abbreviations

aa, amino acids
ACE-2, angiotensin-converting enzyme 2
RBD, receptor binding domain
ORFs, open reading frames
N, nucleocapsid protein
M, membrane protein
E, envelope protein
S, spike glycoprotein
NTD, amino terminal domain
TLR, toll-like receptor(s)
PRR, pattern recognition receptor(s)
IL, interleukin
IFN, interferon
RIG-1, retinoic acid inducible gene 1
MDA5, melanoma differentiation-associated protein 5
NSP, non structural protein(s)
ISG, Interferon-stimulated gene
Ag, antigen
APC, antigen-presenting cell(s)
TMPRSS2, transmembrane serine protease 2
ADCC, antibody-dependent cell-mediated cytotoxicity
DAMP, danger-associated molecular patterns
IM, intramuscularly