Vaccine Development against SARS-CoV-2

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Coronavirus disease (COVID-19) caused by the SARS-CoV-2 virus has been affecting the world since the end of 2019. The severity of the disease can range from an asymptomatic or mild course to acute respiratory distress syndrome (ARDS) with respiratory failure, which may lead to death. Since the outbreak of the pandemic, scientists around the world have been studying the genome and molecular mechanisms of SARS-CoV-2 infection to develop effective therapies and prevention. In this review, we summarize the progressive development of various treatments and vaccines as they have emerged, a year after the outbreak of the pandemic. Initially for COVID-19, patients were recommended drugs with presumed antiviral, anti-inflammatory, and antimicrobial effects that were previously used to treat other diseases. Thereafter, therapeutic interventions were supplemented with promising approaches based on antibodies, peptides, and stem cells.

Keywords: SARS-CoV-2 ; COVID-19 ; cytokine storm ; therapy ; antibodies ; vaccines

1. COVID-19 Outbreak

Since the first cases reported from Wuhan (Hubei Province of China) at the end of 2019, there has been an expansion of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), previously named as novel coronavirus or 2019-nCoV [1], in all continents, including Antarctica [2]. At the beginning of the outbreak, an epidemiological investigation in Wuhan identified an initial association with a seafood market selling live animals [3]. Nowadays, a new data describing molecular and serological evidence of SARS-CoV-2 related coronaviruses in bats occurring in China denote a high possibility of bat-to-human transmission [4].

However, the main means of SARS-CoV-2 transmission overall is from person to person by inhalation of smaller-than-droplet particles (airborne route) [5][6]. From the beginning, non-pharmaceutical recommendations, such as strict hand hygiene, wearing face mask, safe social distancing, and compliance to quarantine, were shown to be effective in controlling the spreading of infection. The virus has been detected also in non-respiratory samples (e.g., blood, stool); however, a role of these biological materials in spreading is unclear [7][8][9]. There were also reports on perinatal transmission route, but whether the transmission has been transuterine, transplacental, or environmental is not determined yet [10][11].

People in their 60s or 70s are generally more susceptible to SARS-CoV-2. Thus, the severity of the disease is positively correlated with age and underlying diseases (hypertension, uncomplicated diabetes, cardiovascular disease, chronic respiratory disease, immune compromised status, cancer, obesity, etc.) [3][12]. The number of children infected by SARS-CoV-2 increased gradually with the rising spread of the epidemic. However, SARS-CoV-2 (like SARS and MERS) was detected in pediatric patients less frequently with milder symptoms and with a better overall outcome than in adults [3].

A broad spectrum of SARS-CoV-2 clinical manifestations in infected patients ranged from mild symptoms that were nonspecific to severe pneumonia with organ function damage [13]. The accompanying symptoms can be grouped into three clusters. The most common respiratory symptom cluster (cough, production of sputum, febrility, etc.), a musculoskeletal cluster (muscle pain, joint pain, headache, and exhaustion), and gastrointestinal (enteric) cluster (vomiting, diarrhea, and abdominal pain) [14]. A pooled analysis of five studies among 817 patients showed that gustatory malfunction (altered taste sensation) was found among 49.8% of COVID-19 patients [15]. Another study has confirmed that anosmia (impaired olfaction) in patients suffering from COVID-19 varied from 33.9 to 68% with female dominance [16][17].

2. The Genome and Structure of SARS-CoV-2

Coronaviruses are single-stranded unsegmented positive-sense RNA viruses with a dimension of 80–120 nm. There are four types of coronaviruses, namely, α-coronavirus, β-coronavirus, δ-coronavirus, and γ-coronavirus [18][19], in which the

genome varies from 26 to 32 kilobases. They belong to the order Nidovirales, the family Coronaviridae, and subfamily Coronavirinae [18]. SARS-CoV-2 belongs to the genus Betacoronavirus [20].

Coronavirus Research Group of the International Committee on Taxonomy of Viruses (ICTV) has determined that a novel coronavirus is affiliated with the SARS virus (SARS-CoV) [21]. Phylogenetic analysis of full-length genome sequences obtained from infected patients showed 79% similarity between SARS-CoV-2 and SARS-CoV [22][23]. As both SARS-CoV and SARS-CoV-2 belong to the category called severe acute respiratory syndrome-related coronavirus, the ICTV assigned the name of this coronavirus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [21]. High genome-wide sequence homology (88–89%) is also found between SARS-CoV-2 and two bat-derived SARS-like coronaviruses, namely, bat-SL-CoVZC45 and bat-SL-CoVZXC21. The sequence homology between SARS-CoV-2 and Middle East respiratory syndrome coronavirus (MERS-CoV) accounts for only 50% [23]. SARS-CoV-2 became the seventh member of the coronavirus family to infect humans [22]. The other coronaviruses are human coronavirus 229E, NL63, OC43, HKUl (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, respectively), SARS-CoV, and MERS-CoV [24]. Variable numbers of open reading frames (ORFs) are found in the coronavirus genome [25]. The SARS-CoV-2 genome was reported to possess 14 ORFs encoding 27 proteins, among which four encode major structural proteins localizing on the surface of SARS-CoV-2, namely, spike surface glycoprotein (S) and matrix protein (M), small envelope protein (E), and nucleocapsid protein (N). These four ORFs are located at the 3′-terminus of the genome [26] (Figure 1A).

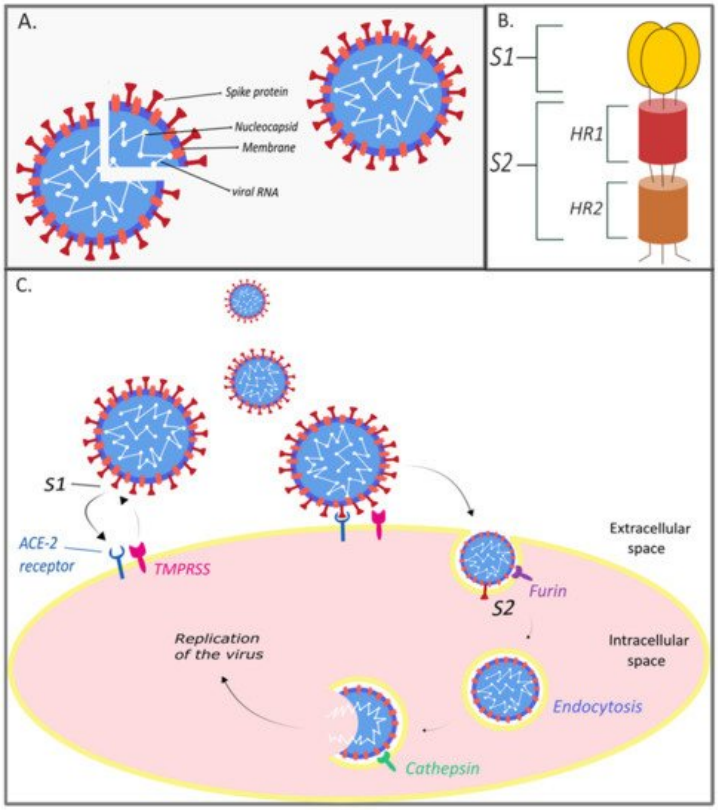

Figure 1. Schematic structure of SARS-CoV-2 and schematic of spike protein and depiction of the SARS-CoV-2 viral entry. (**A**) Schematic structure of SARS-CoV-2. The viral surface proteins including spike protein (S) and membrane protein (M); these proteins are ingrained in a lipid envelope. The single-stranded viral RNA is linked to the nucleocapsid protein. (**B**) Schematic of spike protein. Schematic structure of viral spike protein showing subunits S1 and S2 and domains HR1 and HR2. (**C**) Schematic of SARS-CoV-2 viral entry. S protein targets the host ACE-2 (angiotensin-converting enzyme 2) receptor and enters in the host cell. Note the binding between viral subunits S1/S2 and ACE-2 receptor, as well as cleavage by cell surface protein TMPRSS2 protease. Host cell proteases furin and cathepsin also take part in the cleavage. For the viral particle to enter into the host cell (endocytosis), S protein cleaving into S1 and S2 subunits at S1/S2 cleavage site is essential. This happens either by the serine protease TMPRSS2, or by endosomal proteases Cathepsin B/L [27]. Spike protein can also be cleaved by furin convertases [28]. After the fusion with the host cell membrane, viral genome is released, translated, and replicated. Upon protein assembly, exocytosis takes place, which releases viral particles from the cell. Authors of this article created this figure using Inkscape software; it is not based on any previously published image.

The S glycoprotein is required for binding to receptors on the host cell and plays an essential role in determining host tropism and transmission capacity, mediating receptor binding and membrane fusion [29]. In general, spike protein consists of two functional subunits: S1 and S2 domains. While S1 domain mediates receptor binding, S2 domain is responsible for

cell membrane fusion [30]. Several analyses have shown that SARS-CoV-2 uses cellular angiotensin-converting enzyme 2 (ACE-2) as its receptor for binding to host cells [31][32][33][34][35]. The cleavage site present between S1 and S2 on protein S is proteolytically cleaved by cellular cathepsin L and the transmembrane protease serine 2 (TMPRSMP2) [36]. While TMPRSS2 exposes the surface of the plasma membrane of the host cell, cathepsin L activates S protein in endosomes. Cathepsin L can also compensate the entry into cells devoid of TMPRSS2 [33]. The cleavage site S1/S2 uniquely disposes of the insertion RRAR, located between residues 682 and 685 [32][34][35]. Due to this insertion, S1/S2 can be also pre-cleaved by furin, which reduces the dependence of SARS-CoV-2 on TMPRSS2 and cathepsin L on host cells [32][34]. Considering the fact that S protein can be uniquely pre-cleaved by furin, which is found in almost all tissues and organs, SARS-CoV-2 is able to induce systemic infection. It can therefore be much more infectious compared to other types of SARS-like coronaviruses [35] (Figure 1C).

Although possible mutations were not initially considered, nowadays, much attention has been paid to the health risks of newly discovered variants of the SARS-CoV-2 virus [37]. Both SARS-CoV-2 variants, 501Y.V1 (British B.1.1.7) identified across the UK, except Northern Ireland, and 501Y.V2 (B.1.351) spreading in South Africa, have a mutation (N501Y) in the receptor-binding domain (RBD) of the spike protein, which makes them more transmittable (40–70%) in comparison with other variants [38]. Moreover, the South African and Brazilian (P.1) variants have shown a similar capability of adapting to evade immunity as well as antibody escape [39]. It is still not clear as to whether immunity provided by T cells may protect the organism against these mutations. Therefore, the effect of these mutations on transmission, severity of the COVID-19 infection, and vaccination strategies is currently the subject of numerous scientific studies [37].

It is necessary to note that while the development of coronavirus vaccines and global vaccination is currently underway, the immediate effective treatment that would prevent a serious course of COVID-19 in patients still remains as the main issue. Therefore, various fundamental and experimental therapies involving drugs, antibodies, peptides, or even stem cells with different mechanisms of action have been tested. Since the beginning of the pandemic, safe treatment protocols are gradually being introduced for those confirming efficacy in inhibiting SARS-CoV-2 infection.

3. Vaccine Development

As vaccines are considered the most promising way to eradicate the SARS-CoV-2 virus, several teams are intensively working on vaccine development [40]. Vaccines are being developed with different technologies, some well-known and others completely new for human vaccines, such as peptide and nucleic acid technologies.

Currently, there are two messenger RNA (mRNA) vaccines and two vector vaccines to prevent COVID-19, all authorized by the European Medicines Agency (EMA). The first mRNA vaccine, Comirnaty (BNT162b2), developed by BioNTech and Pfizer, was authorized by December 2020 [41]. In January 2021, the EMA approved the COVID-19 vaccine Moderna (mRNA-1273), which was developed by the National Institute of Allergy and Infectious Diseases (NIAID) in collaboration with Moderna Biotech Spain, S.L. [42]. Both contain lipid nanoparticle (LNPs)-encapsulated mRNA, which encodes the spike protein of SARS-CoV-2 [43]. This technique does not include parts of the virus, only the sequence of spike protein encoded to mRNA. For successful delivery, novel lipid nanoparticles are used to protect the protein sequence. After intramuscular application, the LNPs are removed by myocytes and the mRNA is released and translated to endogenously synthesized spike protein. The mRNA is very sensitive, and therefore is broken down shortly after vaccination. These vaccines activate T cells cytotoxicity as well as B cells response, which ultimately causes strong cellular immunity [43][44][45][46].

The technology of adenovirus-based vectors is an already proven method of vaccine preparation, in a relatively short time, through modification of an adenovirus vector carrier through the insertion of a “gene of interest” such as the code of a spike protein. The Vaxzevria (previously COVID-19 vaccine AstraZeneca/Oxford) is an adenovirus vaccine (ChAdOx) that has been authorized by the EMA since January 2021. It is a chimpanzee adenovirus-vectored vaccine encoding the SARS CoV 2 spike glycoprotein (ChAdOx1-S, ChAdOx1 nCoV-19), manufactured by the Serum Institute of India and SKBio [47]. Results from four clinical trials in the United Kingdom, Brazil, and South Africa showed that Vaxzevria was safe and effective at preventing COVID-19, as well as resulting in robust neutralizing antibody and T-cell responses [48][49][50][51]. In March 2021, with the increase in vaccination across the population, some serious adverse reactions occurred with ChAdOx1 nCov-19 (AstraZeneca) and Janssen (Johnson & Johnson) vaccines. In very rare cases, their use has led to the development of immune thrombotic thrombocytopenia (very similar to heparin-induced autoimmune thrombocytopenia, HIT) caused by anti-platelet factor 4 (anti-PF4) antibodies that activate platelets. These pathological changes may cause unusual clotting such as cerebral venous thrombosis [52][53]. More data on this pathophysiology are therefore crucial for preventing these harmful effects. Nonetheless, EMA issued a statement that the benefits of Vaxzevria continue to outweigh its risks, and the vaccine can continue to be administered.

The COVID-19 vaccine Janssen is another vector vaccine, developed by Johnson & Johnson, that received authorization in the EU in March 2021 [54]. This vaccine is composed of replication-incompetent human adenovirus that encodes a SARS-CoV-2 full-length spike glycoprotein and provokes a similar immune response after vaccination as Vaxzevria.

For the Sputnik V vaccine, which is already registered in more than 55 countries, an EMA rolling review started on the 4th March [55][56]. Unlike the COVID-19 vaccine Janssen, Sputnik V includes two different types of human adenovirus vectors (rAd26 and rAd5), which ensure lasting immunity [57].

All approved vaccines within the EU are safe, while differing in efficacy ranging from 72 to 95%. Most vaccines are given in two doses, except for Johnson & Johnson (Janssen), who state that a single dose will provide protection against the disease. Likewise, they differ in other factors such as time required for full immune response, protection against COVID-19 in the aged and young population, suitability for the elderly, and storage properties.

Expectations from EU-approved vaccines to produce nationwide immunity against COVID-19 as well as identifying their side effects and possible health risks need to be monitored in further large-scale randomized clinical trials.

Several vaccines have been developed and tested in other countries apart from in Europe. India‘s first COVD-19 vaccine Covaxin (Bharat Biotech) is an inactivated vaccine developed using whole-virion-inactivated, Vero cell-derived platform technology. It has demonstrated 81% efficacy [58]. These types of inactivated vaccines have also been developed in China, namely, Synopharm and Sinovac, showing similar efficacy in COVID-19 prevention [59]. American Novavax has developed a unique protein-based vaccine, NVX-CoV2373 (trade name COVOVAX), requiring two doses with an efficacy of 86% (UK variant) and 60% (South African variant) [60].

Vaccination is crucial for achieving a sufficient level of protection against the virus, especially for immunocompromised patients and patients with comorbidities. For this reason, cancer patients who are at an even higher risk of severe COVID-19 infection should be prioritized for vaccination against SARS-CoV-2. These patients are advised to use mRNA vaccines (BNT162b2, Pfizer-BioNTech vaccine), which have a better safety profile and therefore a lower risk of adverse reactions in these patients [61]. However, the efficacy of these human vaccines in these patients is questionable [62]. The BNT162b2 vaccine was shown to be effective and safe in a study of 134 patients and older adults with various frailty and disability profiles, providing protection regardless of their condition [63]. On the other hand, several studies have found lower levels of antibodies in patients with multiple myeloma after the first dose of this vaccine than in the vaccine trials [64][65]. These findings further increase the emphasis of the second dose in cancer patients. However, large prospective and well-designed clinical trials regarding efficacy and safety among immunocompromised patients are necessary.

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