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Review

An overview of COVID-19 and current vaccine studies

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ABSTRACT

The deadly novel coronavirus-2019 (COVID-19) caused by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) virus has been declared a global pandemic by the World Health Organization. The incubation period of the virus, which first emerged in Wuhan, Hubei province of China, is 2 to 14 days. In this review, we discuss the epidemiology, virology, transmission, pathogenesis, immunity and diagnosis of the virus. In addition, vaccine strategies, vaccine stages, vaccine platforms, and various current vaccine studies are reviewed.

Keywords: COVID-19, pandemic, SARS-CoV-2, vaccine.

The coronavirus pandemic (coronavirus-2019 [COVID-19]) caused by the new severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) spreading from Wuhan city of Hubei province, China has rapidly affected the whole world. The World Health Organization (WHO) identified this new virus as the temporary 2019 novel coronavirus (2019-nCoV) on January 12th, 2020.[1] Then, the WHO declared the 2019-nCoV outbreak as an international public health emergency on January 30th, 2020.[2] On February 11th, 2020, the disease caused by 2019-nCoV was officially named COVID-19 by the organization. The International Virus Taxonomy Committee, such as the WHO, named 2019-nCoV SARS-CoV-2 on February 11th, 2020. The virus is still spreading at full speed, its contagiousness remains high, and its curative treatment is not available, yet. The virus threatens all age groups, particularly elderly and immunocompromised individuals.

EPIDEMIOLOGY

The COVID-19 has been rapidly increasing worldwide, since the first case was identified in Wuhan and reached its highest level between February and March. On January 30th, 2020, more than 7,000 cases were reported in China and as of December 11th, 2020, the number of cases has reached 69.6 million worldwide. The United States (US; 15.7 million), India (9.8 million), Brazil (6.78 million), Russia (2.55 million), and France (2.34 million) have the highest number of COVID-19 cases. The number of recovered cases is about 44.9 million and the number of deaths is nearly 1.58 million.

VIROLOGY

Before the COVID-19 outbreak, there were six types of coronavirus which could infect humans: Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV, betacoronavirus HKU1,

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alphacoronavirus 229E, betacoronavirus OC43, and alphacoronavirus. Phylogenetic analysis results show that this virus belongs to the same subgenus Sarbecovirus as SARS.[3]

The SARS-CoV-2 is a coronavirus belonging to the betacoronavirus cluster. In this respect, it is similar to MERS-CoV. The SARS is the third zoonotic coronavirus disease known after COVID-19 and MERS.[1] Zhu et al.[4] confirmed that SARS-CoV-2 was a new strain of betacoronavirus belonging to the Coronaviridae subgenus botulinum. It also shares roughly 82% homology at the nucleotide level with the SARS-CoV, which first emerged in the human population in 2002.[5] As a result of the modeling, COVID-19 and SARS-CoV have a three-dimensional structure in the spike region protein, which is almost the same, and this structure binds to the receptor. In SARS-CoV, this protein has a strong affinity for angiotensin-converting enzyme 2 (ACE2). In addition, the virus enters the cells using the ACE2 receptor, but not the other coronavirus receptors such as dipeptidyl peptidase-4 (DPP-4) or aminopeptidase N (APN).

The SARS-CoV-2 is a (+ single-stranded ribonucleic acid [RNA]) virus with an enveloped and positive sense single-stranded RNA genome (~30 kb).[6] The genome consists of 5' non-structural protein coding regions, which are the two-thirds of the genome, and 3' structural and non-essential accessory protein coding regions. The section encoding two large polyproteins (pp1a and pp1ab from ORF1a and ORF1b) that can be proteolytically cleaved into non-structural proteins (nsp1 to -16) required for the production of new viral genetic material is the first two-thirds of the genome. The remainder carries helper genes which produce virions and change the host response and encode the structural proteins.[7] The R is used to reflect the potential for transmission of a virus, and the R0 value of SARS-CoV-2 was estimated at 2.5 (range, 1.8 to 3.6). The R0 value of SARS-CoV and 1918 flu epidemic is estimated to be between 2.0 and 3.0. It is thought to be 9 for MERS-CoV and 1.5 for the 2009 influenza pandemic.[8] This number is significantly greater than 1. This situation shows that SARS-CoV-2 has a high transmission capacity and, consequently, the ability to cause epidemics.

Transmission

Individuals of all age groups can be infected with the virus and become sick. It can be transmitted by symptomatic patients through coughing and sneezing. Patients infected with SARS-CoV-2 are a source of infection that produces large amounts of virus in the upper respiratory tract during the prodrome period.[5] The disease has been reported to be significantly milder in infected infants, children, and newborns, but they need to be protected as they are carriers and may infect others. During the incubation period, patients show mild symptoms and can continue their routine activities which may increase the spread of the infection. Several researchers have reported COVID-19 infection in newborns.[9] However, transmission from pregnant women has not been conclusively proven. In this case, the possibility of transmission from a pregnant woman to the fetus is low. In mothers infected with COVID-19, no virus was encountered in the umbilical cord blood, breast milk, and amniotic fluid.[10] Although transplacental transmission from pregnant women to their fetuses has not been identified, COVID-19 has been reported as a neonatal disease associated with postpartum transmission.[11]

In addition, asymptomatic carriers can be a source of infection.[12] In this context, asymptomatic patients take the role of carrier and, therefore, they should pay attention to self-isolation and social distance.

The COVID-19 has been shown to infect the gastrointestinal tract according to biopsy and endoscopy results obtained from stomach, rectum, and duodenal epithelial cells.[13] In addition, the ACE2 is rarely expressed in the esophageal epithelium, but abundantly located in the glandular epithelium. The presence of the ACE2 receptor in these tissues supports the entry of SARS-CoV-2 into the cell. The virus can be also detected in feces. In 23% of the patients, it occurs in the stool, although no virus can be detected in the airway samples.[13] These two facts confirm the possibility of fecal-oral transmission of the virus.[13]

The incubation period of the disease ranges from 1 to 14 days with an average duration of three to seven days and, sometimes, it can reach 24 days, which makes screening for infection

difficult. Infected droplets spread one to two meters and can accumulate on surfaces. A person can become infected by breathing these droplets or by touching the nose, eyes, and mouth after touching the contaminated surfaces. The virus can survive on surfaces for days under suitable atmospheric conditions, but can be quickly destroyed by common disinfectants such as hydrogen peroxide and sodium hypochlorite.[14]

Pathogenesis

Zhao et al.[15] found that ACE2 acts as a receptor for SARS-CoV-2. The ACE2 types I and II are expressed in the alveolar epithelial cells of human lung. In their study, a significantly higher ACE2 levels were found in the alveolar cells of men than women. In addition, excessive ACE2 expression was observed in the alveolar cells of Asians, compared to African Americans and whites. Binding of SARS-CoV-2 to the ACE2 receptor causes excessive expression of ACE2, leading to alveolar cell damage. Damage to the alveolar cells can lead to many systemic reactions, pneumonia, respiratory failure, and subsequent death.[15] It has been associated with an overgrowth of inflammatory cytokines such as interleukin (IL)-7, IL-2, IL-10, inducible protein (IP)-10, granulocyte colony-stimulating factor (GCSF), macrophage inflammatory protein (MIP)-1A, MCP-1, and tumor necrosis factor-alpha (TNF-α).[16]

The COVID-19 binds to receptors and enters cells. Glycoproteins in the envelope spike virus bind to cell receptors in the form of ACE2 in COVID-19. The virus, then, breaks down the genetic material of the cells and synthesizes the required protein, creating new virions. After the virus enters the cell, the viral RNA genome is released into the cell cytoplasm. The viral genome is, then, copied. Glycoproteins in the nascent viral envelope enter the membrane of the cells of the Golgi or endoplasmic reticulum.

The plasma membrane and vesicles containing virus particles combine to release new viruses. In SARS-CoV, protein S has been reported as an important determinant of virus entry into host cells. With the entry of SARS-CoV into cells, the viral membrane forms fusion with the plasma membrane of the cell. In this process, the S2 protein is involved in the proteolytic cleavage process, aiding to the membrane fusion process. Another mechanism other than the membrane fusion which mediates the entry of SARS-CoV into host cells is clathrin-independent and clathrin-dependent endocytosis.[17]

When the virus enters the cell, the viral antigen is presented to antigen presenting cells (APCs). Viral antigen presentation is dependent on the major histocompatibility complex (MHC) Class I molecules. The MHC Class II molecules also contribute to this process. Antigen presentation stimulates cellular and humoral immune responses mediated by T and B cells. In the humoral immune response, immunoglobulin (Ig)G and IgM against SARS-CoV are formed. The latter disappears at the end of the 12th week and IgG can persist for a long time. A study including recovered patients showed that, after four years, the SARS-CoV-specific CD8+ and CD4+ memory T cells could be found, but the number gradually decreased in the absence of antigens.[18]

In summary, the virus spike protein is a glycoprotein with a trimeric structure that can specifically recognize ACE2 by its receptor binding domain (RBD). The conformational changes that occur as a result of RBD/ACE2 interactions first cause fusion of cellular lipid and viral membrane and, subsequently, cell infection.

Immunity

A study conducted in China showed the difference in the immunological profile between severe and mild cases of COVID-19.[19] In this study, in severe COVID-19 cases, lymphocyte count was found to be lower, neutrophil and leukocyte ratios were found to be higher, and eosinophil, monocyte, and basophil percentages were found to be lower. In severe clinical cases, proinflammatory cytokines such as IL-6 and IL1, IL-8, TNF-α and infection markers such as ferritin, C-reactive protein and procalcitonin were also found to be higher. In severe cases, lower helper and regulatory T cells were seen.[19]

In another case report of a COVID-19 patient with acute respiratory distress syndrome, CD8 and CD4 T lymphocytes were found to reduce.[20] The CD8 T lymphocytes contained high concentrations of cytotoxic granules (30.5% positive for granulicin and perforin, 64.2% granulicin positive, and 31.6% positive performance). An increase in the

proinflammatory Th17 CCR6 + concentration was also seen.

Diagnosis

Chest computed tomography (CT) scanning is performed in cases of pneumonia. In addition, liver function, kidney function, albumin, and blood gas analysis (BGA), blood glucose measurement, and sensitivity tests are performed to identify possible bacterial causes, if there are multiple infection bacteria. Commonly, reverse transcription-polymerase chain reaction can be performed by taking a nasopharyngeal swab from the patient.

Considering symptoms of the patients, Guan et al.[21] evaluated 1,099 cases in their study.[21] In infected patients, 87.9% had fever, 67.7% had cough, 38.1% had fatigue, 33.7% had sputum, 18.7% had shortness of breath, 3.7% had diarrhea, and 5.0% had vomiting. In addition, lymphopenia was found in 82.1% and abnormalities in chest CT scans were found in 96% patients. In this study, the mortality rate was 1.36%.

In the study of Huang et al.,[22] 73% of 41 patients with SARS-CoV-2 infection were men, and six of the patients had hypertension, eight had diabetes, and six had cardiovascular disease. In this study, a 15% case fatality rate was found.

Vaccine strategies and phases

Researchers have benefited from the fact that both SARS-CoV-2 and SARS-CoV use human ACE2 (hACE2), which acts as a receptor in the host cell, and that the genetic structures of these viruses are 82% similar to each other.[23] In addition, based on the previous studies on the MERS virus, they developed vaccination strategies. The main goals are to develop vaccines and provide immunization to prevent contamination.

It is mandatory to comply with the Good Clinical Practice (GCP) principles in all clinical studies. There are four phases in the vaccine development studies:

In Phase I, the aim is to evaluate safety. In this context, the dose range is determined, its pharmacokinetic properties are established, and the product is studied at the level of absorption, distribution, metabolism and excretion. A series of gradually increasing single dose applications are made. The study including 20 to 80 volunteers usually takes 1 to 1.5 years.

In Phase II, the main objective is to evaluate safety and efficacy. About 100 to 300 volunteers are recruited and the study takes approximately two years. The effectiveness of the drug is established in patients, its side effect profile is investigated, dose-response data are collected, and the most appropriate application method of the drug is investigated.

Phase III requires 1,000 to 3,000 volunteers and studies in this phase take about three to four years. The clinical efficacy and side effects of the drug are evaluated in the wider population in randomized, double-blind, multi-national, and multi-center settings.

Phase IV studies are conducted after the launch of the product and are post-marketing surveillance studies. These studies are carried out with thousands of volunteers and can take many years. The main goal of these studies is to collect the long-term safety data. Side effects not occurring during clinical trials are reported in Phase IV studies.

The (S) protein facilitates binding to the ACE2 host receptor in SARS-CoV-2; therefore, (S) protein is the primary determinant of host pathogenicity and transmissibility.[24] It is also considered to be the first target for vaccine design, as it is the main target for neutralizing antibodies. Excluding S protein, other viral proteins such as E protein, N protein, and non-structural protein 16 (NSP16) can be also considered the targets for vaccine development. In this context, the fact that the coronavirus N gene has a lower mutation and has more conserved sequences, compared to other genes, can be another useful target for the vaccine.[25]

Standardization, quality control, and critical efficacy testing of vaccines are required to determine the level of immune responses generated. Then, the vaccine needs to be further tested. Volunteers can be infected as a dangerous way to do this. Some vaccine developers use the Goldilocks approach. In this approach, volunteers are infected with enough viruses which do not tire their immune systems.

Vaccine platforms

Among the vaccine platforms used against SARS-CoV-2, examples are inactivated whole virus vaccines, live attenuated vaccines (LAVs),

inactivated virus vaccines, subunit vaccines, RNA-based vaccines, and viral vector-based vaccines. Each method has different merits and demerits and are summarized below.

The virulence of live viruses is reduced in the live attenuated vaccines (LAV) method. It usually gives long-term immunity and induces toll-like receptors (TLRs) and B cells of the innate immune system, including CD4 and CD8 T cells. There is a possibility that nucleotide substitution occurs in the replication of the virus and results in recombinants after vaccination. In addition, LAVs can infect individuals with compromised immune systems and revert to their lethal genetic form.

Inactivated virus vaccines are safer than LAVs and have been tested for many diseases, including SARS-CoV. The additives used in this platform can increase immunogenicity and, thus, the vaccine can be administered at a lower dose. However, in this method, the immunogenic particle integrity must be preserved. They may need more than one dose to build up the immune memory. Of note, the adjuvants used may induce an undesirable inflammatory response.

Subunit vaccines have less side effects, since the virus particle does not contain live components. However, immune responses become weakened over time.

Viral vector-based vaccines have been widely used for MERS-CoV. These vaccines stimulate both humoral and cellular immune system against antigen. As a limitation, however, they may cause cancer by integrating the viral genome into the host genome. Also, if the host has been previously exposed to this viral factor, the immune activity may be less.

Plasmids containing deoxyribonucleic acid (DNA) against viral antigens are produced in the DNA-based vaccine type based on *in situ* production of the target antigen. Artificial DNA is heat-resistant. There is no risk of infection. However, it can be integrated into the chromosomal DNA of the recipient, inducing the response of B and T cells and, eventually, weakening the immune response of the host.

For RNA vaccines, the translation of the messenger RNA (mRNA) occurs in the host cell cytosol and, thus, there is no risk of the viral genome integrating into the host genome. It can stimulate both humoral and cellular immune systems. However, if it is applied directly to cytosol, it may increase antigen expression.

According to the information published by the WHO as of December 10th, 2020, there are 52 vaccine candidates which have been clinically evaluated and 162 vaccine candidates which have been pre-clinically evaluated worldwide.

Current Vaccine Studies

Some of the most discussed vaccine developers that are making progress in phase stages are summarized below.

BioNTech-Pfizer

For the vaccine developed by BioNTech-Fosun Pharma-Pfizer, which is one of the most discussed vaccines in the world, this is an RNA-based vaccine and is administered in two doses with a dose interval of 21 days. The vaccine does not contain the SARS-CoV-2 virus. In this vaccine method, two lipid nanoparticles were formulated against SARS-CoV-2, and nucleoside-modified RNA (modRNA) was evaluated as vaccine candidates. The BNT162b1, one of the vaccine candidates, encodes the SARS-CoV-2 RBD and is trimerized by adding a T4 fibritin foldon domain to increase the immunogenicity of the virus through the multivalent display.

BNT162b1 induces interferon (IFN)-2 and IL-producing CD8+ cytotoxic T cell and CD4+ type 1 helper T (Th1) cell responses. It appears to elicit neutralizing antibodies and RBD-binding IgG often with mild side effects (e.g., injection site pain and fatigue). It is an important feature that the BNT162b1 vaccine produces both cellular and humoral antiviral induction.[26] The other vaccine candidate, BNT162b2, encodes the full-length spike protein of SARS-COV-2, which is modified by two proline mutations involved in the conformational change of prefusion.[27] On the other hand, by analyzing the toxicity and immune response of BNT162b2, this vaccine candidate is immunogenically stable with a favorable reactogenicity profile.

Moderna

Moderna, another vaccine developer, developed an mRNA-based vaccine (mRNA-1273) consisting of a sequence-optimized mRNA encoding spike glycoprotein encapsulated in lipid nanoparticles.[28]

While Pfizer's vaccine must be stored at -70° before delivery, Moderna's vaccine can be stored at -20°. Vaccine is administered in two doses and there are 28 days between each dose. In this vaccine, the genetic information of the coronavirus is injected into a non-virulent viral vector to create viral proteins that mimic the coronavirus. Recent studies have shown that the vaccine can stimulate the production of antibodies that neutralize the virus, with CD8 + T-cell responses seen to occur, when a higher dose (100 µg) of the vaccine is given. A few days after vaccination, non-severe side effects such as shivering, tiredness, injection site pain, fever, and myalgia may occur.

AstraZeneca

AstraZeneca's candidate vaccine strain ChAdOx1-S uses the non-replicating viral vector platform and is a replication defective chimpanzee adenovirus vector vaccine that expresses the SARS-CoV-2 full-length spike glycoprotein gene. A single dose of vaccine protects the lower respiratory tract from infection by inducing cellular immune responses.[29] Induction of IFN gamma (IFNγ) T-cell responses and humoral responses characterized by anti-spike glycoprotein IgG were observed in most recipients after the first dose of vaccine. Humoral immune results increased, after the second dose of vaccine. Among the first results obtained from the clinical trial of ChAdOx1, the vaccine caused minor side effects in the form of fatigue and headache and could induce a T-cell response with a strong antibody.

Sinovac Biotech

The vaccine developed by Sinovac Biotech is an inactive vaccine. The vaccine is administered in two doses with 14 days between each dose. In the Phase II study, measurement was made by enzyme-linked immunosorbent assay and anti-RBD antibodies were formed. In 92% of individuals who received the vaccine in the first 14 days, neutralizing antibodies emerged in 97.4% after 14 days of the second dose. Currently, the researchers have carried out the Phase III study in Indonesia.[30]

Gamaleya

The vaccine made by the Gamaleya National Research Center is a two adenovirus 5-based vaccine containing the recombinant adenovirus serotype 26 (rAd26) vector and the recombinant adenovirus serotype 5 (rAd5) vector.[31] It consists of two injections and the second is given after 21 days to further induce the immune response. The CD4+ and CD8+ T cells immune responses were observed in all volunteers, and these immune responses peaked on Day 28. The results show that the vaccine provides 92% protection. The fact that the results of the Phase III studies have not been published yet is, however, not convincing in terms of its efficacy and safety.

Johnson & Johnson

Johnson & Johnson continues to develop Ad26-based vaccines for viruses such as human immunodeficiency virus, Zika, and Ebola. A double-blind, randomized, placebo-controlled Phase III trial has been initiated for the replication-defective Ad26.COV2.S vaccine developed for COVID-19. (For 60,000 participants aged ≥18). This vaccine given intramuscularly without adjuvant induces a strong neutralizing antibody. It provides protection against SARS-CoV-2 in Rhesus macaques between the ages of 6 and 12 years.[32]

CanSino Biologics

Using adenovirus type 5 vector, CanSino Biologics has developed a viral vector vaccine that expresses the SARS-CoV-2 spike protein and introduces the protein to the body. The reports published in the Lancet are promising, as only one dose of the vaccine has been shown to induce a significant immune response without documented serious side effects.[33] Of note, responses to vaccines can be misleading, as adenovirus-based vectors are based on natural viruses that some individuals may have previously been exposed to. Another concern is the dose of the vaccine. If high doses are required, inflammation may occur as a result of the acceleration of the immune system. To prevent this, the vaccine should be administered with a low-dose adjuvant.

The TLRs directly activate APCs and, thus, can generate humoral and cellular immune response, which is our goal for the fight against COVID-19. It is possible to speculate that TLR is potentially an adjuvant, since it increases TH1 responses.[34] In addition, the Bacillus Bacille Calmette-Guérin (BCG) vaccine can strengthen immunity against COVID-19, as it contains different types of live bacteria. In one study, COVID-19 deaths

decreased in patients having BCG vaccine. The BCG can also contribute to the immune system that combats against SARS-CoV-2 by bridging adaptive immunity and innate immunity.[35] As a result, there was a decrease in COVID-19 deaths with BCG vaccination. Based on this, in countries with BCG vaccination such as South Korea and Japan, there are fewer COVID-19 infections than countries without BCG vaccination, such as US, Italy, and the Netherlands.

DISCUSSION

The SARS-CoV-2, which was declared as a pandemic, could not be controlled worldwide and caused many deaths. The main reason for this is that the R0 value of the SARS-CoV-2 virus is much higher than the SARS-CoV, MERS-CoV, and influenza viruses, leading to rapid spread of the virus and causing individuals to become infected. In addition to being the source of infection of symptomatic patients, asymptomatic patients also posed a threat to the community. Thus, the infection spread increasingly. The expression of the ACE2 receptor not only in the alveolar cells of the lungs, but also by the rectum and duodenal epithelial cells facilitated the processing of the virus by entering the cells. Indeed, the fact that the virus survives on contaminated surfaces for a while made it easier for individuals to become infected. In this context, the importance of personal hygiene and cleanliness has once again become important. The mortality rate found by Guan et al.[21] was 1.36%, while the mortality rate found by Huang et al.[22] was 15%. This difference may be due to different sample numbers or the presence of viral variations. As a result of the studies, patients with chronic diseases such as diabetes and heart disease and the elderly, i.e., immunocompromised patients, have this disease severely. Additionally, it is thought that the first infection occurred in late November 2019 or earlier, based on factors, such as the incubation period of the virus. Based on the genetic similarity of SARS-CoV-2 to SARS-CoV and MERS-CoV viruses, S protein-targeted vaccines are being developed currently. Although vaccines introduced by different developers induce immune system responses, the rates of protection from infection vary. The reasons for this may be that the vaccine candidates are created by different methods, have different immunogenic balance or have different reactogenicity. In addition, vaccines using adenovirus may produce a strong antibody response, but may be safer and more effective in combination with adjuvants.

The COVID-19 vaccine needs to be accepted by every member of the society. If even a few individuals refuse to be vaccinated, the society would be negatively affected. In addition, storage conditions of vaccines should be suitable for all countries. The vaccine, which should be kept in the cold chain, should be properly protected in the US and African countries. One of the most important issues is the cost of vaccines; the vaccine must be affordable and sufficient for the whole world.

In conclusion, in this review, an overview of the SARS-CoV-2 virus, which adversely affects the whole globe in all aspects, is given based on the studies conducted to control and prevent the pandemic. In addition, a brief information about current vaccination studies is provided. For the SARS-CoV-2 pandemic, it is important to eliminate the transmission routes, isolate the source of infection, address infection-related transmission risks, and accelerate the treatment and diagnosis of the cases. Until the society is vaccinated and immunized, a particular attention should be paid to social distance, self-isolation, personal hygiene, and wearing masks. Due to the rapid spread of the SARS-CoV-2 virus, individuals in the risk groups need additional self-protection.

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