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Review Article

SARS-CoV-2 Potential Therapeutic and Immunologic Targets - A Review

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Abstract

This review of the literature aims at pondering over some of the characteristics shared among the three coronaviruses causing severe respiratory syndromes in humans, the natural evolution of the COVID-19 infection, and the different clinical and diagnostic needs of each phase. In addition, we consider the experimental development of vaccines against SARS-CoV-2, with focus on the two studies already initiating phase-3 clinical trials, as well as the many safety issues and questions yet to be answered, and the necessary criteria to be established by the regulatory agencies concerning which segment of the population may actually benefit from vaccination.

Keywords: *SARS-CoV-2; COVID-19; MERS-CoV*

Background

Human infecting coronavirus share several phylogenetic common features in their capsid structure and spike glycoprotein as well as in their proliferative mechanisms, which are potential therapeutic targets for the repositioning of known medications, development of new ones, and of vaccines as well. Out of the seven coronavirus strains (two alpha and five beta strains) that cause respiratory diseases in humans, the SARS-CoV, the MERS-CoV and the SARS-CoV-2 are the ones responsible for the epidemics of severe acute respiratory syndromes of 2002/2003, 2012 and 2019/2020, respectively, whereas the others are mostly associate with common colds [1,2].

The SARS-CoV and SARS-CoV-2 strains share close molecular and genetic similarities such as their dependence on receptors for angiotensin-converting enzyme 2 (ACE2) to trigger its access to the host's cells, wherein they release their single-strand RNA to be replicated and translated into structural and functional proteins to form thousands of new virions. The structural proteins that form the spikes, the viral envelop, the nuclear capsid, and the membrane are being tested as epitopes in the development of vaccines in several research centers worldwide. The viral RNA is first translated in the cell cytoplasm into two polyproteins (pp1a e pp1ab) which are cleaved into functional proteins by two proteases [1]. SARS-CoV and SARS-CoV2-2 also share a proteinase structure that controls the RNA replication and transcription through two overlapping replicase enzymes [3]. Other characteristic of these coronaviruses is that they require a low pH medium for viral protein translation. Therefore, existing medications that elevate cellular pH and/or block the ACE2 receptor or disturb the ribosomal translation of viral proteins have a potential benefit against these pathogens.

Development of new drugs or the repositioning of existing ones should take into consideration the natural history of COVID-19, which comprises three different phases, each requiring different clinical management strategies. The stage one or early infection lasts between 4

and 6 days and it is when viral replication rapidly occurs, under the attack of the innate immunity while the adaptive immune response is still being mounted. Symptoms usually are coryza, anosmia, dry cough, fever around 99.6 F or higher, migraine, with or without diarrhea. Blood analysis will show lymphopenia, mildly increased LDH, augmented D-dimer and increased prothrombin levels.

Our clinical experience and that of colleagues working at the Prevent Senior hospitals network in São Paulo city, SP, Brazil, have shown that when dry cough initiates, lung CT-scan already shows that ~20 to 25% of the lower and peripheral pulmonary area is already affected by ground-glass-like lesions and is gradually losing gas exchange capacity, which suggests that lung damage initiates at the first phase of the disease. Prevent Senior physicians have publicly informed that they adopted lung CT-scans as the golden standard for fast COVID-19 diagnosis because it allowed the early onset of treatment and medical follow up still in stage 1, even before lab assay confirmation. Available assays, by the way, are not satisfactorily specific in the first weeks of infection and false negative results may be misleading and may cost lives. For instance, RT-PCR may yield false negative results in up to 30% of patients and SARS-CoV-2-specific IgM showed 69% sensitivity, whereas IgG sensitivity was 93.1%, based on PCR positivity [4,5]. However, IgG may be undetectable in the first two weeks of symptomatic disease. This may be related to the fact that the inflammatory phase of COVID-19 peaks up after the adaptive immunity is activated and starts mounting its attack, which occurs approx. 96 hours after the onset of inflammation by the innate immunity (e.g. on the fourth - fifth day). In elderly patients the adaptive response is usually less effective and may take longer to be mounted, due to the decreased production of T lymphocytes as well as the reduced IgG response to some antigens, which leads to increased infection severity.

Lungs CT-scans showing ground-glass-like lesions are feasible during the first phase, once dry cough is installed. Systemic and local thrombotic risk is elevated from the second phase onwards and changes in the lung arteries and alveolar vessels are also detectable by CT-scans imaging [6].

Studies combining the antimalarial chloroquine or hydroxychloroquine with macrolide antibiotics were reported to be effective in some controlled observational studies during the initial symptomatic phase of COVID-19 (first four-five days), but gradually less beneficial as the second phase progresses towards the acute third phase, when other clinical effects of pulmonary damage and the high levels of inflammatory factors, such as C-reactive protein, IL-6, LDH, troponin, D-dimer, ferritin, NT-proBNP, promote systemic toxicity and circulatory disorders. However, high doses of hydroxychloroquine may be toxic and unnecessary, since daily doses of 400 mg during 5 or 7 days have showed efficacy in the initial phase [7-11]. Patients with the polymorphic profile of low metabolizers of medications, such as the majority of the Asians, Native Americans, and Native Brazilians, should receive even lower doses of those antimalarials because they tend to accumulate in tissues higher doses than the complete metabolizers [12].

The stage 2 comprises the onset of respiratory shortness and hypoxia progression (PaO_2/FiO_2 < 300 mmHg) as alveolar lesions spread through the lungs up to > 50%, leading to decreased gas exchange. High levels of hepatic transaminase enzymes are detected in this phase as well as low levels of procalcitonin, which is typical of viral infections. Higher levels of C reactive-protein (CRP > 26.9 mg/L) is an important biomarker of vascular damage and disease progressing towards phase three, with increased inflammatory activity. The introduction of dexamethasone, interleukine-2 and 6 inhibitors, heparin, and other supportive interventions are necessary to control the cytokine storm and prevent thrombotic events and septic shock [13]. Patients over 50 years of age with vitamin D3 deficiency (< 0.25 ng/L) [14], chronic pulmonary comorbidities, smokers as well as those with cardiomyopathies, obesity, deep vein thrombosis, or high blood pressure may rapidly progress to phase 3 and are at high risk of death. Phase three is characterized by the need of intensive care, mechanical assisted breathing, septic shock, multiple organ failure, and cardiac arrest.

Vaccines development

Vaccines are tools for passive immunization and each approach aims to expose the immune system to a particle or protein sufficiently antigenic to cause an effective immune response and the formation of an immune memory against that microorganism. Vaccines develop-

ment therefore begins with the search for the ideal antigen or epitope, which should cause enough immunogenicity to promote immune adaptive memory but without causing unacceptable toxicity such as happens with superantigens. Superantigens are microbial or viral antigens that are able to bind to nearly all T cells bearing a variable region of the β chain at the T-cell antigen receptor. An example of a superantigen is the toxin that causes the toxic-shock syndrome by activating a larger than normal number of helper T cells. Superantigens bind directly to class II MHC complexes (e.g. without the mediation of antigen-presenting cells) and interacts with the beta chain of T-cell receptor in many T cells simultaneously. Therefore, they induce a massive stimulation and expansion of T cells population, followed by deletion and anergy [15].

More than 100 vaccines for COVID-19 are currently in experimental development worldwide, both in universities and in pharmaceutical laboratories. Several technologies are being explored and some of these have never before been used in vaccines licensed for humans. At least four research centers are already conducting Phases 2/3 clinical trials on human volunteers to verify their safety, efficacy, effect duration, and tolerability. Other groups are conducting initial clinical experiments on animal models, such as mice and several species of monkeys [16].

Types of antigens under testing are: 1, the virus itself either inactivated or weakened; 2, viral vectors bearing a selected SARS-CoV-2 antigen (epitope), with the viral vector either being replicating or non-replicating; 3, proteins or proteins subunits similar to the virus - in this case, only the external outer coat of the virus, containing the spikes, is used; and 4, DNA vaccines or nucleic-acid vaccines, using a messenger RNA encoding an specific viral protein (such as the spike protein), inserted in human cells wherein they translate the viral protein and trigger the antigenic response. This approach was never used for human-intended vaccines so far [16].

When the chosen epitope is a viral mRNA or a gene encoding an immunogenic protein such as the spike of coronavirus, it must be delivered into the host's cells by another virus that acts as a carrier. Since all viruses introduce their genetic material in the cells of a host, they can be altered and recombined to prevent pathogenicity and be used as an efficient manner of introducing the antigen of another virus in the host's cell. The advantages of this approach are: (a) high gene transduction; (b) highly specific delivery of genes to target cells; and (c) induction of robust immune responses, (d) and increased cellular immunity. The main risk of viral vectors is the development of high immunogenicity and genotoxicity.

The most studied candidates to viral vectors are the several adenoviruses strains which cause human gastroenteritis, acute respiratory syndrome, and pharyngo-conjunctival fever. Recombinant adenovirus vectors are replication-defective due to E1A and E1B viral gene deletions and highly efficient in gene transduction and transgene expression but also have a broad tropism. They can infect both dividing and non-dividing cells and might cause toxicity in cells of other organs, such as the liver. Other disadvantage of using adenovirus vectors is that humans were widely exposed to approximately 50 different serotypes and already have immune memory of the main structural epitopes of these viruses [17].

A 2003 study with Rhesus macaques successfully immunized the animals with adenovirus vectors transfecting the spike S1 fragment of SARS-CoV and produced antibodies and T-cell proliferation against the N protein [18]. A 2005 similar study using mice also obtained immunization against SARS-CoV [19] as well as two others, using adenovirus coding the S-protein of SARS-CoV in ferrets [20,21]. The problem with adenovirus vectors in humans is to find a serotype for which we are still naïve. One candidate vector is the chimpanzee adenovirus (ChAdOx1) [22] which showed persistent immunity and safety in humans in a 2019 trial developing the MERS001 vaccine against MERS-CoV. One single injection was effective to immunize simians against the MERS-CoV [23] and the phase 1 trial in humans showed safety and both innate and acquired immune responses in the three study arms tested with different doses (5×10^9 viral particles; 2.5×10^{10} viral particles; and 5×10^{10} viral particles) [24].

Safety measures in vaccine development include the previous testing in animal models before testing in humans. Vaccinated animals will be later infected with the virus to check the efficacy of the vaccine. The main safety concern is to prevent induction of acute disease

which may occur when the vaccinated individuals develop a more severe form of the disease than those who have never been vaccinated. Decades ago, vaccines developed against another coronavirus, feline infectious peritonitis virus, increased the risk of cats developing the virus-related peritonitis [24]. Similar phenomena have been observed with other viruses in animal studies, including the coronavirus that causes SARS-CoV [25].

Regulatory Agencies should continue to require vaccine researchers to verify and report potentially harmful responses found in animal studies. The evaluation of existing antibodies to any strain of coronavirus in humans should be carefully considered before enrolling them in safety tests. For instance, Moderna sent an experimental messenger-based RNA (mRNA) vaccine to the US National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland, for testing in a clinical trial. The mRNA-based platform for vaccine administration demonstrated that this technology is safe in humans, but the vaccine against COVID-19 that Moderna was developing was not safe [26].

Phase 2/3 clinical anti-SARS-CoV-2 vaccine trials

Two research groups, one in the United Kingdom ("Oxford vaccine") and the other in China ("Wuhan vaccine") have already tested anti-SARS-Cov-2 vaccines in phases 1 and 2 clinical trials and are now recruiting volunteers or initiating phase 3 trials with a large number of participants (> 3,000 subjects).

The Oxford study

The UK vaccine used as viral vector the simian replication-deficient adenovirus (ChAdOx1) (above mentioned) transfected with the SARS-CoV-19 spike protein [27].

Study design in brief

A total of 1,077 healthy adult volunteers of both genders (49.8% females ChAdOx1 nCoV-19 and 50.2% males) were included between April 23 and May 21, 2020, in the phase 1-2 single-blind, randomized controlled trial of recombinant ChAdOx1 nCoV-19, compared to a licensed meningococcal group A, C, W-135, and Y conjugate vaccine (MenACWY; Nimenrix, Pfizer, UK), as control. Objectives: to assess the efficacy as measured by cases of symptomatic virologically confirmed COVID-19; and safety of vaccination with 5×10^{10} viral particles of ChAdOx1 nCoV-19 either in single-dose or in two-dose regimen, as measured by the degree of adverse events. After randomization, 543 participants received ChAdOx1 nCoV-19 and 534 received the MenACWY vaccine.

Results of the Oxford phase 1-2 clinical trial

Immune responses to ChAdOx1 nCoV-19: Antibodies against SARS-CoV-2 spike protein peaked by day 28. Participants who received a single dose had median 157 ELISA units (EU), IQR 96 - 317; n = 127 and remained elevated to day 56 (119 EU, 70 - 203; n = 43). Participants who received a booster dose increased to a median of 639 EU (360 - 792) at day 56. The ChAdOx1 nCoV-19 vaccination resulted in marked increases in SARS-CoV-2 spike-specific effector T-cell responses from day 7, peaking up from day 14 on and maintaining until day 56.

Systemic reactions at day 1: (a) Fatigue was reported by 70% of those taking paracetamol and by 71% of those without paracetamol. (b) Headaches were related by 68% of those without paracetamol and by 61% of those who took paracetamol. Other systemic adverse reactions reported: muscle ache 60% participants without paracetamol and 48% with paracetamol; malaise 61% without paracetamol and 48% with; chills 56% without and 27% with paracetamol; feverish felling: 51% without and 36% with paracetamol. As for fever intensity, in the ChAdOx1 nCoV-19 group 18% of participants without paracetamol and 16% with paracetamol reported a temperature of at least 38°C and 2% without paracetamol had a temperature of at least 39°C. Local reactions: Sensation of mild pain at the injection site

was reported by 83% of those who did not take paracetamol and by 77% of those who did. The severity of both systemic and local adverse reactions was highest on day 1 after the inoculation; and the majority of the adverse events were mild or moderate in severity.

Conclusion

The study concluded that the efficacy, safety and the control of adverse effects with paracetamol supports the recruiting to a phase 3 clinical trial including senior individuals with comorbidity, health professionals, and groups at high risk to compare between the one-dose and two-dose regimens. Phase 3 is currently initiating in Brazil and in the UK aiming at enrolling volunteer health professionals, plus citizens of every walk of life and ethnicity, with accrual already over 5,000 participants and growing.

The Wuhan vaccine

Non-replicating Adenovirus serotype 5 (Ad5) transfected with the full-length SARS-CoV-2 gene that encodes the spike protein was used as vector and compared to a placebo containing the vaccine excipients but no SARS-CoV-2 particles [28].

Study design in brief

Randomized, double-blind, placebo-controlled, phase 2 trial of the Ad5-vectored COVID-19 vaccine was done in a single center in Wuhan City (Hubei province, China). Objectives: to assess effectiveness, ideal dose, immunogenicity, safety, and adverse effects of the Ad5-vectored COVID-19 vaccine. After screening, 508 volunteer participants were considered eligible and randomized at a 2:1:1 ratio between the three study arms. The mean age of the participants was 39.7 years (SD 12.5; range 18 - 83), with 309 (61%) individuals aged 18 - 44 years, 134 (26%) aged 45 - 54 years, and 65 (13%) aged 55 years or older across the treatment groups. Two different vaccine doses (either 1×10^{11} or 5×10^{10} viral particles per mL) were given in the arms randomized to receive actual vaccine, while the third arm received the placebo vaccine. Blood samples were taken from participants at day 0 immediately before vaccination, and at days 14 and 28 post vaccination for the measurement of specific antibody responses against the receptor binding domain (RBD) using ELISA kits (Beijing Wantai BioPharm, Beijing, China). The number of participants randomized to the three study arms were 253 in the arm receiving 1×10^{11} viral particles; 129 received 5×10^{10} viral particles; and 126 received the placebo vaccine.

Results of the Wuhan phase 2 trial

Immune response: Ad5-vectored COVID-19 vaccine induced detectable RBD-specific ELISA antibody responses from day 14 onwards, with GMTs of 94.5 (95% CI 80.5 - 110.8) and 85.1 (66.0 - 109.7) in the 1×10^{11} and 5×10^{10} viral particles dose groups, respectively. The RBD-specific ELISA antibodies peaked at day 28 up to 656.5 (575.2 - 749.2) in the arm inoculated with 1×10^{11} viral particles and up to 571.0 (467.6 - 697.3) in the arm that received 5×10^{10} viral particles. Neutralizing antibodies responses to live SARS-CoV-2 were induced by both dose ranges at day 28, with GMTs of 19.5 (95% CI 16.8 - 22.7) and 18.3 (14.4 - 23.3) in participants in the 1×10^{11} and 5×10^{10} viral particles dose groups, respectively. The participants who had low pre-existing anti-Ad5 antibodies had the highest RDB-specific ELISA levels of antibody and neutralizing antibodies - approximately twice as higher than those with high pre-existing anti-Ad5 immunity 266 (52%). Age was also a negative factor on RDB-specific ELISA antibody and neutralizing antibody responses. Participants age 55 or older had relatively low antibody and neutralizing antibody responses in the both arms receiving the vaccine. Ad5-vectored COVID-19 vaccine induced significant SARS-CoV-2 spike glycoprotein-specific IFNγ-ELISpot responses in 227 (90%, 95% CI 85-93) of 253 participants receiving the 1×10^{11} viral particles dose, and in 113 (88%, 81 - 92) of 129 participants receiving the 5×10^{10} viral particles dose at day 28.

Adverse events: During the 14 days after the vaccination, 183 (72%) out of the 253 participants in the 1×10^{11} viral particles dose arm, and 96 (74%) out of the 129 participants in the 5×10^{10} viral particles dose arm have reported at least one solicited adverse reaction. The most common systemic adverse reactions reported were: (a) fatigue: 42% of the arm inoculated with the higher dose; and 34% in

the lower dose arm; fever: 32% and 16% respectively; headache: 29% and 28%, respectively. The local reaction reported was pain in the injection site, in 57% of patients in the lower dose arm and 56% in the higher dose arm. Degree of adverse event reported by the majority were mild or moderate, except by 9% (n = 24) who received the lower dose of the vaccine (1×10^{11} viral particles) who reported grade 3 adverse events, such as fever in 8% (n = 20) and in 1% of those in the higher dose arm (5×10^{10} viral particles) The grade 3 reactions disappeared within 72-96 hours and without medication. Other grade 3 reactions in both arms were vomiting (3%), diarrhea (16%), muscle pain (33%), joint pain (23%), lack of appetite (16%), nausea (13%), and oropharyngeal pain (14%).

Study Conclusion

Wuhan investigators concluded that the results obtained not only expanded their knowledge on the immunogenicity and safety of the Ad5-vectored COVID-19 vaccines, but also provided a rational foundation for the testing of the Ad5-vectored COVID-19 vaccine at 5×10^{10} viral particles a phase 3 clinical trial including different age groups and using the vaccine dose 5×10^{10} viral particles to validate its effectiveness in healthy adults.

Final Thoughts

So far, these two studies seem to be the more promising ones, given the careful trial design, the number of volunteering participants, and the results shown in the clinical phases already accomplished. With different viral vectors (ChAdOx1 vs. Ad5), both studies concentrated on S-protein epitopes. It is too early to define which will show higher effectiveness and longer immunity persistence, besides lesser grades of adverse reactions in a larger population. Other ongoing approaches under investigation may also bring new effective strategies in the near future. As a matter of fact, at the exact moment this review is being published, Astra Zeneca is putting on hold its Phase 3 Trial, due to a severe reaction case, yet to be clarified, in one of the participants. The two Russian vaccines also raises safety concerns due to several study limitations. Caution and safety should not become a lesser priority because of the epidemic, since long-term effects of such a hurry may be worse than the initial disease.

Finally, sound criteria should be exercised by both vaccine developers and regulatory agencies concerning the identification of those specific groups who could actually benefit from the vaccination. For instance, should patients with D3 deficiency receive immunization? Should those already actively immunized, through environmental exposure to SARS-CoV-2, receive passive immunization? Which age range should be immunized? Patients with life-threatening chronic diseases such as cancer, autoimmune disorders, age-related immune anergy, or immune suppression should be included in vaccination programs? Meanwhile, let us continue saving lives by starting the early treatment of COVID-19 patients, in the first three-five days of symptoms, using repositioned off-label well-known antiviral drugs [9-11], which, when administered at medium to low doses for short periods (5 to 7 days) have shown to be safe and effective.

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