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Review

Vaccines for COVID-19: perspectives from nucleic acid vaccines to BCG as delivery vector system

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Abstract

This article discusses standard and new disruptive strategies in the race to develop an anti-COVID-19 vaccine. We also included new bioinformatic data from our group mapping immunodominant epitopes and structural analysis of the spike protein. Another innovative approach reviewed here is the use of BCG vaccine as priming strategy and/or delivery system expressing SARS-CoV-2 antigens.

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The pandemic Coronavirus Disease 2019 (COVID-19) is the third global threat mediated by betacoronaviruses within this century. These enveloped viruses were thought to be restricted to animals until the first outbreak in 2002, where Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) infected around 8000 people reaching a fatality ratio of 9.6% [1]. Again in 2012, Middle East Respiratory Coronavirus (MERS-CoV) was the cause of an endemic in Middle Eastern countries, affecting almost 2500 people with approximately 35% of fatality ratio and outbreaks outside the region [1]. Less than a decade later, SARS-CoV-2 spread COVID-19 worldwide, so far with more than 18,847,261 confirmed cases and 708,469 deaths (data updated on 08/06/2020, available at John Hopkins Coronavirus Resource Center, https://coronavirus.jhu.edu/map.html). Currently, the only way to deal with this disease is through supportive care to treat the symptoms and mandatory isolation to slow down the transmission, that includes even those not affected. The course of COVID-19 and other coronaviruses diseases outbreaks can overload worldwide health systems and have severe implications in lives lost and economics, underscoring the need for effective vaccines.

SARS-CoV-2, together with SARS-CoV and MERS-CoV, belong to the cluster of betacoronaviruses, presenting large positive-sense RNA genomes. Cellular infection initiates when the spike glycoprotein binds to its cellular receptor, angiotensin-converting enzyme 2 (ACE2) for SARS-CoV and SARS-CoV-2 [2], or the dipeptidyl peptidase 4 (DPP4) for MERS-CoV [3]. After membrane fusion, the viral RNA genome is released into the cytoplasm. The replication cycle begins with the translation of non-structural proteins (nsps), whose main function is to form the replication-transcription polyprotein complex. Afterwards, a nested set of subgenomic mRNAs is transcribed into structural and accessory proteins [4,5]. A unique feature of coronaviruses is the exoribonuclease function of nsp14 that provides proofreading function to the polyprotein complex and contributes to the maintenance of the large RNA genome [6,7]. The subgenomic mRNAs encode the four structural proteins spike (S), envelope (E), membrane (M) and nucleocapsid (N), as well as proteins that interfere with the host innate immune response [8–10]. At the 5’ prime of SARS-CoV-2 genome we can find ORF1a (polyprotein pp1a) and ORF1b (Fig. 1). The ORF1a encodes 2 important proteases: (i) the papain-like and (ii) the 3C-like protease, also called Mpro [11–13].
During SARS and MERS, the activation of the RLRs RIG-I and MDA5 retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) [25]. This process results in the excessive production of inflammatory cytokines and chemokines followed by gene expression programs indicative of development of an adaptive immune response [37,38]. By contrast, those patients who died maintained high levels of ISG-encoded proteins, CXCL10/IP-10 and CCL2/MCP1, associated with low or absent production of spike-specific antibodies, which suggests that severe disease is related to the lack of a switch from an innate to an acquired immune response [19]. The infection with SARS-CoV induces an adaptive immune response against predominantly the structural antigens of the virus rather than nsps [39,40]. The S protein acts as a major antigen for both humoral and cellular acquired immunity [41]. Consistent with this, most peptides identified as CD4+ or CD8+ T cell epitopes are derived from the S protein, although there are also peptides from N and M proteins [42]. This observation may be related to the distribution and physiology of the structural proteins. The S protein is more exposed to the host immune system and influences virus entry; the M protein is the most abundant protein in the virion, and N protein is conserved among different coronaviruses [42]. T cell responses often target highly conserved internal proteins and promote long-term protection. Memory CD4+ T cells are more numerous at sites of infection than CD8+ T cells and have multiple roles in initiation and maintenance of efficient immune response, while cytotoxic CD8+ T cells (CTLs) eliminate infected cells [43–45]. CD4+ T cells protect against lethal disease through rapid local IFN-γ production and induction of neutralizing antibodies. These cells can also facilitate
CD8+ T cell responses by stimulating migration of innate cells and CD8+ T cell mobilization [46]. CTL mediated protection through increased production of IFN-γ and cytotoxicity [40]. The antibody response is crucial for preventing viral infection and to reduce viral titers during ongoing infection. A subset of these antibodies is defined as neutralizing antibodies due to their capacity of blocking the entry of the virus into a healthy cell by binding to surface viral epitopes. Besides that, enveloped viruses can be eliminated through complement activation or antibody-mediated opsonization [47]. During SARS-CoV infection it seems that neutralization of viral invasion and elimination by phagocytic macrophages are the main mechanisms of protection, with neutralizing IgGs playing a major role [48]. Memory B cells provide long-lasting protection in SARS in association with cellular immune responses [49]. However, the protective ability of T cells is desirable since SARS-CoV antibodies levels decline rapidly after recovery. SARS-CoV-specific memory T cells persisted for at least 6 years in patients who have recovered from the disease while memory B cell response was not detected by this time [50].

The early research on SARS-CoV-2 infection identified some peculiarities that should be considered for medical interventions, such as vaccine development. Some COVID-19 patients secreted excessive IL-4, IL-13 and IL-10, which may be an indicator of inflammatory suppression via T-helper 2 or regulatory immune responses [16]. In most cases lymphopenia occurs, with significant reduction in T cells and NK cells numbers in severe cases. This clinical outcome is associated with functional exhaustion of cytotoxic lymphocytes [16,51]. Moreover, the high CXCL10/IP-10 production during SARS-CoV-2 infection is a sign of virulence since high and persistent levels of this chemokine are associated with death of patients in all recent epidemic CoVs infections. Some patients with COVID-19 develop a defective immune response that may lead to accumulation of immune cells in the lungs and overproduction of pro-inflammatory cytokines/chemokines, causing severe damage to the lung and a systemic pathogenesis [52]. A simple and direct approach that has been investigated to combat rapidly the COVID-19 is passive immunity through the use of plasma from convalescent patients [53]. Even though promising, the outcomes of this therapy are unpredictable due to variability of sera from different patients. Antibody-based therapies and vaccines should dedicate great effort to safety evaluation given the reports of antibody-dependent enhanced SARS-CoV entry and antibody-mediated lung injury during SARS in experimental models [54–56]. However, the viral peptides that induce protection can be identified and distinguished from the detrimental ones through epitope design [55]. Vaccine strategies include live attenuated and inactivated whole virions, nucleic acids (DNA and RNA) and recombinant proteins, most of them focusing on the highly immunogenic aspect of the spike protein.

2. The spike protein: the main target of host immune response

It is known that SARS-CoV-2 enters the host cells by the fusion of viral and cellular membranes [57–61] with the densely glycosylated spike protein. The S protein is a class I viral fusion protein and an important target for antibody neutralization and vaccine development. The role of glycosylation in camouflaging immunogenic protein epitopes has been studied for other coronaviruses. High viral glycan density and local protein architecture can influence the trafficking of recombinant immunogens to germinal centers [62]. Fusion S proteins of SARS-CoV-2, SARS-CoV and MERS-CoV are examples of viral proteins that are widely glycosylated [63]. The spike protein is comprised of three protein domains (Fig. 2) [12]. They participate in the cell recognition process by binding to host cell receptors and facilitating membrane fusion [58]. Analysis shows around 69–87 N-linked glycosylation sites identified on the surface of each trimeric peak in SARS-CoV-2 S protein, and approximately 22 N-linked glycosylated amino acids per domain (PDB ID 6VSB) [62]. The receptor binding sites formed by amino acids and glycan residues are a common feature of viral glycoproteins, as observed on SARS-CoV S and MERS-CoV S (respectively, PDB ID 5X58 and PDB ID 5X59) [64]. This structural glycosylation has functions in viral pathology such as mediating protein folding and stability, besides shaping viral tropism [65]. Thus, these glycosylation sites show selective pressure, as they facilitate evasion of the host immune system by protecting specific epitopes that induce antibodies or T cell responses [66,67].

S protein allows the virus to attach on the target cell and fuse membranes, and it is presented as a trimeric structure on the viral surface [68,69]. During the infection phase, S proteins are cleaved into two subunits: (a) receptor binding subunit, S1, and (b) membrane fusion subunit, S2. It uses the N-terminal region as a signal sequence to access the endoplasmic reticulum of cells and this region is highly N-glycosylated. The S1 subunit of S protein has a receptor binding domain (RBD) composed of a central subdomain (core) and a connection motif for the receptor. The central subdomain has four antiparallel sheets, connected by alpha-helices, and stabilized by 3 disulfide bridges. The protein’s S2 subunit is also similar to that of SARS-CoV and is responsible for the fusion of membranes from severe conformational changes [69,59,70]. S2 has two regions of heptad repeats (HR). During the fusion process, S2 dissociates from S1 and the HR1 and HR2 regions and form a 6-helix bundle (6-HB) structure, exposing a hydrophobic fusion peptide inserted in the host membrane and allowing the membrane to approach the virus for fusion.

Recent experimental data support biophysical and structural evidence that the glycosylated S protein of SARS-CoV-2 binds to the ACE2 receptor with greater affinity than that of SARS-CoV [12]. Hence, the atomic level understanding of these interactions is important for the structural and biophysical elucidation of the initial virus infection process in human cells. By screening the experimentally-determined SARS-CoV-derived B cell and T cell epitopes in the immunogenic structural proteins of SARS-CoV, many authors identified a set of B cell and T cell epitopes derived from the spike and the nucleocapsid proteins that map identically to SARS-CoV-2 proteins. As no mutation has been observed in these identified epitopes among the 120 available SARS-CoV-2 sequences (as of February 2020), immune targeting of these epitopes may potentially offer protection against this novel virus. These results provide a screened set of epitopes that can help guide experimental efforts towards the development of vaccines against SARS-CoV-2. The identification of critical residues involved in receptor binding is important for the development of vaccines and inhibitor targets [63]. These studies provide regions to be modeled and investigated either experimentally or theoretically by computer simulations [42]. For this purpose, Briell et al. compared the interaction between ACE2 and peak SARS-CoV-2 S protein with that of other pathogenic coronaviruses using classical molecular dynamics (MD) simulations [71]. The authors observed that SARS-CoV and SARS-CoV-2 have comparable binding affinities. However, the complex formed by the interaction and fusion between SARS-CoV-2 and ACE2 contains a greater number of contacts between the amino acid residues of the side chains of the proteins. These data imply an evolution due to mutations to increase cell recognition and have implications for therapeutic strategies [72]. Veeramachaneni et al. evaluated through computational analysis the structural changes caused by specific mutations, hot spots binding residues and their interactions between the SARS-CoV-2 S RBD protein receptor and ACE2 [73]. We performed molecular dynamic simulations of the
complexes. The major amino acids involved in the binding identified by interaction analysis after simulations, include the Glu 35, Tyr 83, Asp 38, Lys 31, Glu 37, His 34 amino acid residues of the ACE2 receptor and Gln 493, Gln 498, Asn 487, Tyr 505 and Lys 417 residues in the SARS-CoV-2 S protein RBD. By locating these amino acid residues, the authors propose that blockers can be designed to inhibit binding and interrupt the entry of the SARS-CoV-2 virus into host cells (Fig. 3).

By using a computational approach, the RBD region of the SARS-CoV-2 S protein was explored to identify various immunodominant epitopes for the development of diagnostics and vaccines. B cell linear epitope probability and MHC binding affinity were determined for all sequential peptides with a single amino acid displacement by our group, using an updated version of methods previously described [74]. The results obtained here could also help us to understand the SARS-CoV-2 surface protein response towards T- and B-cells. Mapping of predicted B and T cell epitopes indicates that the most probable B cell epitopes are located throughout the RBD region of Spike protein (Fig. 4). In the region of amino acids 450–525 there are also two regions where sequential 15-mers are predicted to have high affinity binding for many human MHC II DRB alleles. Within the region of amino acids 475–500 there is also a region of sequential 9-mers predicted to bind to multiple MHC class I alleles. This indicates that the RBD region of Spike from SARS-CoV-
2 is most likely to elicit a strong antibody response due to the number of B cell epitopes with associated T cell help predicted and may also elicit CTLs.

3. Vaccine strategies against SARS-CoV-2

The spread of COVID-19 challenged the world to accelerate research in companies and universities in the search for a safe and effective vaccine against SARS-CoV-2 [75]. At the time of writing (August, 2020), at least 165 teams are working on different projects towards the same goal of developing a COVID-19 vaccine. The development process has been optimized with at least 26 groups already starting to test their vaccine candidates in clinical safety trials and many others are testing their candidates in cells and animals. Researchers are investing in different strategies and technologies, but most of these novel approaches have not been extensively tested for safety and do not have large-scale manufacturing capacity to produce the high number of doses needed. For this reason, the many candidates still in the race are creating real possibilities and new knowledge in vaccine design [76,77].

Twenty-six vaccine candidates are in clinical trials, six of which are in final clinical tests (phase 3) using different designs and vaccine strategies (Table 1). Among the SARS-CoV-2 vaccines under development are whole virus vaccines, nucleic acids vaccines, viral vector vaccines and protein-based vaccines. Virus vaccine are the most common platform used for other diseases. SARS-CoV-2 could be used as an inactivated or live attenuated virus that conserves most of the virus antigens. There are currently three vaccines based on inactivated virus already in phase 3 clinical trials, including one vaccine from SINOVAC (NCT04456595) and two from Sinopharm (ChiCTR2000031781). Instead of using the whole virus, many studies use protein subunit vaccines including antigens with strong immunogenicity, most focusing on the spike protein or only the receptor binding domain. Another protein-based vaccine strategy consists of virus-like particles (VLP) that mimic the SARS-CoV-2 structure on the surface of a non-replicative empty virus shell lacking genetic material [78,76,79]. Several virus (e.g., vesicular stomatitis virus (VSV), influenza, measles and adenovirus) could also be engineered as replicative or non-replicative recombinant vector expressing coronavirus S protein. A live-attenuated recombinant vesicular stomatitis virus (VSV) expressing the Ebola glycoprotein (VSV-EBOV) successfully completed a phase III clinical trial and was shown to be safe and immunogenic against the Ebola virus [80]. The same strategy was used to make an attenuated VSV recombinant expressing the SARS-CoV spike protein (VSV–S) able to control a challenge with SARS-CoV and stimulating neutralizing antibody in mice [81,82]. The ChAdOx1 nCoV19 vaccine developed at the University of Oxford is a very promising candidate that uses a replication-deficient chimpanzee adenovirus to deliver SARS-CoV-2 antigen protein. A single dose of ChAdOx1 nCoV19 has protected six rhesus macaques from pneumonia caused by SARS-CoV-2, pushing the vaccine to a phase 3 (ISRCTN89951424) clinical trial [83,77]. Another phase 2 candidate from CanSino Biological Inc./Beijing Institute of Biotechnology uses non replicating Adenovirus type 5 as a vector based on the same platform strategy used for Ebola (Phase 2 – ChiCTR2000031781) [77,84].

Another strategy is focused on nucleic acid manipulation to build DNA or RNA vaccines. A genetic construct coding for specific antigen(s) can be easily synthesized in DNA or RNA and inserted into human cells to generate many copies of the immunogenic virus protein. DNA vaccines are typically generated by a plasmid DNA containing eukaryotic expression elements that encode one or more antigens. The plasmid contains components that allows growth and selection of the vector in bacteria, such as Escherichia coli, followed by a purification process. The eukaryotic expression cassette contains a 5’ promoter, the gene of interest and a 3’ polyadenylation (poly A) signal, important for nuclear export, translation and stability of the transcript mRNA [85–87]. DNA vaccines are stable but have the challenge of needing to cross two cellular membranes before entering the nucleus, and may also bring the risk of vector integration in the human genome. The most common route of DNA vaccine administration is intramuscular or intradermal injection. However, DNA vectors alone generally lead to relatively low immunogenicity. Therefore, different delivery methods have been developed to improve DNA uptake, including the gene gun, needle free injection (jet injection) and in vivo electroporation [88,86].

The mRNA vaccines manufacturing process is essentially chemical, comprising an in vitro transcription from a linearized DNA template, then removing the template by digestion with DNases to get a purified mRNA. mRNA comprises a 5’ cap, a 5’ untranslated region (UTR) (leader RNA), the coding sequence with a stop signal, a 3’ UTR, and a poly(A) tail. mRNA enters into the
cytoplasm as a template to be translated making multiple copies of the antigen(s) protein(s) [87]. Various mRNA vaccine platforms have been developed to render the synthetic RNA sequence more translatable, stable and non-toxic. The use of “naked” mRNA is not recommended because RNA is highly unstable under physiological conditions, due to the extracellular ribonucleases which catalyze RNA hydrolysis and due to the hydrophilicity and strong conditions, due to the extracellular ribonucleases which catalyze RNA hydrolysis, and due to the hydrophilicity and strong conditions, due to the extracellular ribonucleases which catalyze RNA hydrolysis.

Strategies for optimizing mRNA vaccines includes: synthetic cap analogues and capping enzymes to stabilize mRNA and increase translatable, stable and non-toxic. The use of mRNA is not taken up efficiently by the cells. DNA vaccines were first tested in 1993, showing protective immunity against influenza in mice [99]. In the same year, a liposome-entrapped mRNA vaccine in mice was shown to induce virus-specific cytotoxic T lymphocytes response [89]. After decades, new techniques and formulations have improved, so SARS-CoV-2 candidates could possibly be the first licensed human nucleic acid vaccine.

### Table 1

| Platform vaccine | Consortium | Candidate vaccine | Clinical Stage | Clinical trial register |
|------------------|------------|-------------------|----------------|------------------------|
| Inactivated      | Sinovac    | Adsorbed COVID-19 (inactivated) | Phase 3 | NCT04456595 |
| Wuhan Institute of Biological Products/Sinopharm | Inactivated novel coronavirus pneumonia (COVID-19) | Phase 3 | ChiCTR2000034780 |
| Beijing Institute of Biological Products/Sinopharm | Inactivated novel coronavirus (2019-CoV) | Phase 3 | ChiCTR2000034780 |
| Institute of Medical Biology, Chinese Academy of Medical Sciences | Inactivated SARS-CoV-2 | Phase 1/2 | NCT04470609 |
| Bharat Biotech | Whole-Virion Inactivated SARS-Cov-2 Vaccine (BBB152) | Phase 1/2 | NCT04471519 |
| Non-Replicating Viral Vector | University of Oxford/AstraZeneca | ChAdOx1 nCoV-19 | Phase 3 | ISRCTN89951424 |
| CanSino Biological Inc./Beijing Institute of Biotechnology | Recombinant Novel Coronavirus (2019-nCoV) Vaccine (Adenovirus Vector) | Phase 3 | ChiCTR2000031781 |
| Janssen Pharmaceutical Companies | Ad26Cov51 | Phase 1/2 | NCT04436276 |
| Gamaleya Research Institute | Gam-COVID-Vac Lyo | Phase 1 | NCT04437875 |
| Moderna/NIAID | mRNA-1273 | Phase 3 | NCT04470427 |
| BioNTech/Fosun Pharma/Pfizer | BNT162b1 | Phase 3 | NCT04368728 |
| Arcturus/Duke-NUS | ARCT-021 | Phase 1/2 | NCT04480957 |
| Imperial College London | LNP-nCoVsaRNA | Phase 1 | SRCTN17072692 |
| Curevac | CvnCoV | Phase 1 | NCT04449276 |
| People’s Liberation Army (PLA) Academy of Military Sciences/Walvax Biotech. | SARS-CoV-2 mRNA | Phase 1 | ChiCTR2000034112 |
| DNA | Inovio Pharmaceuticals/International Vaccine Institute | INO-4800 | Phase 1/2 | NCT0436410 |
| Osaka University/AnGe/Takara Bio | AG0301-COVId19 | Phase 1/2 | NCT04463472 |
| Cadila Healthcare Limited | Novel Corona Virus-2019-nCoV | Phase 1/2 | NCT044826352 |
| Genexine Consortium | GX-19 | Phase 1/2 | NCT04453839 |
| Protein Subunit | Anhui Zhifei Longcom Biopharmaceutical/Institute of Microbiology, Chinese Academy of Sciences | Recombinant New Coronavirus | Phase 2 | NCT0466085 |
| Novavax | SARS-CoV-2 s (COVID-19) Nanoparticle | Phase 1/2 | NCT04368988 |
| Clover Biopharmaceuticals Inc./GSK/Dynavax | KBP-COVID-19 | Phase 1/2 | NCT04473690 |
| Vaxine Pty Ltd/Medytox | SCR-2019 | Phase 1 | NCT04459908 |
| University of Queensland/CSL/Seqirus | Monovalent Recombinant COVID19 Vaccine (COVAX19) | Phase 1 | NCT0453852 |
| Medicago Inc. | SARS-CoV-2 Scalm Protein Subunit Vaccine | Phase 1 | ACTRN12620000674932p |
| Medigen Vaccine Biologics Corporation/NIID/Dynavax | Coronavirus-Like Particle COVID-19 | Phase 1 | NCT04450004 |
| Medigen Vaccine Biologics Corporation/NIID/Dynavax | MVC-COV1901 | Phase 1 | NCT04487210 |

* Source: WHO - DRAFT landscape of COVID-19 candidate vaccines (07/31/2020).
knowledge and comparison among well-known virus (SARS-CoV and MERS) and SAR-CoV-2 provided a screened set of epitopes candidates that can help guide experimental efforts and accelerate the development of specific SARS-CoV-2 vaccine [67,103,104].

The first full genome sequence of SARS-CoV-2 was made public in January 2020, and within a few days the U.S. National Institutes of Health (NIH) and Moderna’s infectious disease research team finalized the sequence for mRNA-1273. Two months later they started a safety phase I clinical trial to test a lipid nanoparticle (LNP) dispersion containing an mRNA that encodes for the prefusion stabilized spike protein 2019-nCoV and they are now conducting the final clinical tests (Phase 3 - NCT04470427). Inovio Pharmaceutica also focused on the S protein to build a DNA plasmid vaccine administered intradermally followed by electroporation that is being tested on healthy volunteers (Phase 1/2 - NCT04336410). So far, there are 15 projects involving DNA vaccines and 19 involving RNA vaccines (Table 1) [78,77]. Nucleic acid vaccines are promising, but there is still no licensed manufacturing platform. However, recent improvement in nucleic acid vaccine stability and protein translation efficiency combined with large financial investments may bring a new disruptive vaccine technology.

4. BCG as a priming strategy or delivery system for SARS-CoV-2 vaccine

*Mycobacterium bovis* BCG (Bacillus Calmette-Guerin) vaccine against tuberculosis (TB) is the most widely used vaccine in the world, has an excellent safety standard and has been shown to be also an effective adjuvant inducing cellular immunity in animals and humans. BCG is a live attenuated vaccine produced in more than 40 sites around the world using different substrains (Connaught, Danish, Glaxo, Moreau, Moscow, Pasteur and Tokyo) which are not identical in efficacy, safety and immunogenicity. The World Health Organization (WHO) adopted requirements for BCG vaccine in 1965 and still maintains lyophilized seed of the vaccine strains to prevent deviation from the original BCG [105–108]. BCG is a slow-growing organism and provides low-level and persistent antigenic exposure, favoring the induction of a long-lasting cellular and/or humoral T-cell immune response with just one dose [109,110]. In addition, BCG vaccine induces protection in neonates, has high stability, well-established large-scale production and low cost.

Recombinant BCG (rBCG) maintains all BCG’s characteristics. rBCG has also been investigated as a vehicle for antigen delivery strategy against different pathogens. It is possible to construct rBCG strains expressing different levels of viral, bacterial or parasitic pathogens antigens, resulting in the activation of cellular and/or humoral immune response depending on the vector and antigen [111]. BCG vaccine expressing HIV immunogens demonstrated its efficiency in activating the production of cytokines and T cell responses in mice, showing a strong potential as an integrative vaccine against HIV-1/TB or as a priming associated with other virus vector boost vaccines [112–115]. Different rBCG strains expressing specific antigens have induced protection against the challenge with the respective pathogen, including *Borreella burgdorferi*, *Streptococcus pneumoniae*, *Leishmania major* and *Plasmodium falciparum* [116–120]. Currently, rBCG studies are also focused on improving the BCG vaccine efficacy against tuberculosis [121,122].

In addition to inducing a specific anti-TB immune response, BCG vaccination appears to have other effects that have been associated with decreased infant mortality and a lower prevalence of other infections [123–125]. The revaccination with BCG in adults or the elderly can reduce respiratory tract infections [126,127]. This may be due to the trained innate immunity mechanism caused by metabolic changes, epigenetic reprogramming, cytokines releases, monocyte activation and improved host immune response after BCG vaccination [128–131]. The COVID-19 pandemic raised the question whether BCG vaccine could offer protection or be used as a tool in the fight against SAR-CoV-2. The phenomenon has recently been correlated with the possibility that countries where vaccination for TB is mandatory would (so far) have a lower incidence of COVID-19 cases. However, as we are comparing very different cultures and people, it is difficult to exclude other variables that could confuse the analysis and some results are contradictory. Another aspect that needs to be taken into account is that the persistence and immunostimulatory properties of BCG strains differ and it could lead to a variable trained immunity response [132–138]. A recent study compared infection rates and proportions of severe COVID-19 in two similar populations in Israel, comprising one group with individuals born during the 3 years before and other group born 3 years after cessation of the Universal BCG vaccine program, resulting in no statistically significant difference-between BCG-vaccinated vs unvaccinated group [139]. To better investigate the correlation between BCG vaccine and SARS-CoV-2 infection, large clinical trials, including one already started in Australia [https://clinicaltrials.gov/ct2/show/NCT04327206] and others in different countries, propose to test BCG vaccination in health professionals naturally exposed to COVID-19 infection to determine whether heterologous protection exists or not. The heterologous effect of BCG vaccination remains a vast field for research.

Our group is working in collaboration with national and international institutes to develop a new vaccine strategy using rBCG to express the immunodominant epitopes in the RBD region of the S protein from SARS-CoV-2 (Fig. 4) that can lead to immune system activation synergistically with BCG recognition. In addition, another interesting strategy would be to use a BCG vector to express other agonists that activate important innate immunity pathways involving type I IFN, which play an important role in the anti-viral response and in the recruitment of lymphocytes [140–142]. The concern that people already vaccinated with BCG could mount an immune response against the vector, preventing it from delivering the spike protein antigen into human, does not seem to be relevant given the satisfactory results with other studies involving rBCG persistence in the host. Another concern involves the release of IL-6 and other inflammatory cytokines that could aggravate COVID-19 pathology. However, previous studies related to other diseases suggests that BCG could lead to a decrease in viremia at the beginning of the SARS-CoV-2 infection, before a possible cytokine storm happens, thus preventing a systemic inflammation and severe disease [131,143]. Netea and collaborators suggest that BCG vaccine could be used at the beginning of a pandemic as a booster of the host defense, even if effective for a limited period of time, it might contribute reducing SARS-CoV-2 spread and help fight the pandemic until a specific vaccine against COVID-19 can be developed [135,134]. BCG vaccine can be explored as a priming strategy together with other virus specific vaccines, as a potent adjuvant and/or as a delivery system for SARS-CoV-2 proteins in host cells. The exploration of rBCG’s potential for the development of new products can help to solve this and other epidemic outbreaks that may arise.

5. Conclusions

During SARS-CoV-2 infection, immune responses are believed to be essential for viral infection clearance and immunological memory. However, they also cause collateral damage to the lung tissue that can be detrimental and even fatal in some cases. This is a comprehensive review that has focused on host immune responses to SARS-CoV-2 infection, potential epitope targets for vaccine
development and different vaccine strategies from live viral vectors, protein-based vaccines, nucleic acid vaccines and the use of BCG as a potential delivery system to boost antiviral response via trained immunity. Additionally, we added original data on prediction of B cell and T cell epitopes on RBD region of spike protein, structural comparison analysis between the SARS-CoV-2, SARS-CoV and MERS CoV S proteins and interaction of SARS-CoV-2 RBD with ACE2 receptor. Here, we discussed the hypothesis that BCG vaccination might be a potent preventive measure against SARS-CoV-2 infection and/or may reduce COVID-19 disease severity. One critical vaccine target is to raise antibodies directed to the SARS-CoV-2 spike protein and its receptor-binding domain, the component required for virus binding to its host cell entry receptor ACE2. Given the immediate threat of the SARS-CoV-2 pandemic, vaccine trials should be designed and started as pragmatic studies with feasible primary end points that can be performed rapidly and that could provide results in a short period. Due to limitations in vaccine development, randomized controlled trials are needed to provide the highest quality proof that these vaccines can protect against COVID-19. Additionally, we also must recognize that there are potential safety issues that could slow the clinical development path and testing. Since, there is a desperately urgent need to develop strategies that restrain SARS-CoV-2 and limit the pandemic, worldwide efforts are gathered to move forward with all these vaccine candidates already in clinical testing and development.

Declaration of competing interest

EJH is an employee and equity holder in ioGenetics LLC. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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