Current vaccine technology with an emphasis on recombinant measles virus as a new perspective for vaccination against SARS-CoV-2

Rim Jbeli¹ · Awatef Jelassi²

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Abstract

The novel coronavirus disease 2019 (COVID-19) that emerged in China has spread to more than 212 countries to date. COVID-19 can cause serious acute respiratory syndrome (SARS). Therefore, research advances on the associated SARS-coronavirus-2 (CoV-2) may enable the scientific community to establish effective vaccines to prevent SARS-CoV-2 infections by increasing understanding of viral pathogenesis. Measles virus (MV) expressing SARS-CoV-2 spike protein (S) represents a promising class of biotherapeutic agents to combat this virus. The potential of such recombinant viruses has been well recognized for the treatment of many diseases. We summarize and review herein a potential therapeutic intervention strategy against COVID-19 infection based on MVSchw2-SARS-S and MVSchw2-SARS-Ssol with the aim of assessing the suitability of recombinant MV as a potential new candidate SARS vaccine. Such analysis of COVID-19 pathogenesis could also help establish appropriate therapeutic targets for the production of specific antiviral agents against this newly emerged pathogen.

Keywords Coronavirus disease 2019 (COVID-19) · Measles virus (MV) · Recombinant viruses · MVSchw2-SARS-S · MVSchw2-SARS-Ssol

Introduction

COVID-19 has caused extreme alarm among people around the world. Various patients with a respiratory disease of unknown cause appeared in the capital city of Hubei Province, central China, in December 2019. Time revealed that a novel coronavirus SARS-CoV-2 was linked to this respiratory disease that included severe acute metabolic syndrome (SARS) (Xu et al. 2020). SARS-CoV-2 has recently appeared in humans, yet numerous human coronaviruses (HCoVs), including HCoV-HKU1, HCoV-OC43, HCoV-NL63, and HCoV-229E, are known to have spread in humans for years (Corman et al. 2018). Patients with respiratory disease associated with the coronavirus present fever

(above 38°C) and symptoms such as breathing difficulties, temporary conditions, and frosted-glass appearance of lungs (Huang et al. 2020).

Coronaviruses are responsible for 15–30% of colds and respiratory or gastrointestinal infections in humans, as well as cats [feline infectious peritonitis virus (FIPV)] (Niels 2009), poultry [infectious bronchitis virus (IBV) for birds] (Cavanagh 2007), mouse [mouse hepatitis virus (MHV)] (Raamsman et al. 2000), pig [transmitted gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), porcine respiratory coronavirus (PRCoV), and hemagglutinating encephalomyelitis virus (HEV)] (Chen et al. 2005), and cattle [bovine coronavirus (BCoV)] (Ann 1993). In addition, antibodies and cellular immunity can kill infected cells. The mortality rate ranges between 3% and 6% (Johns Hopkins University 2021).

Coronaviruses are spherical enveloped viruses (between 60 and 220 nm in diameter), and their structure is, in part, hypothetically, composed of a helical nucleocapsid within a capsid having a decahedral structure, surrounded by the same membranous shell. Their name derives from their crown-like appearance on electron microscopy (Marra et al. 2003). Coronavirus is a positive-sense single-stranded RNA virus (30 kb) that multiplies in the cytoplasm of the host cell;

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✉ Rim Jbeli
jbeli.rima91@gmail.com

¹ Department of Biology Sciences, Faculty of Sciences of Bizerte, University of Carthage, 7021 Zarzouna, Bizerte, Tunisia

² Laboratory of Biochemistry, LR 99 ES 11, Faculty of Medicine, University Tunis Elmanar, Tunis, Tunisia

The 5' part of the genome encodes a cap structure, while the 3' end encodes a poly-A tail (Wu et al. 2013). The virus has an encased structure that presents peplomeric structures called spicules. The genome contains the following open reading frames (ORFs) from the 5' to 3' end: ORF1a and ORF1b corresponding to replication complex proteins, and ORF-S, ORF-E, ORF-M, and ORF-N corresponding to the structural proteins S, E, M, and N (Kopecky-Bromberg et al. 2007). It also includes ORFs with an unknown role, comprising the region between ORF-S and ORF-E, the overlapping region between ORF-M and ORF-N, and the ORF-N region (Ramaiah and Arumugaswami 2020). In addition, the coronavirus includes three membrane proteins (S, E, and M) and a nucleocapsid protein (N).

Protein S forms a glycoprotein (200–220 kDa) membrane with the shape of spines or “spikes” emerging from the surface of the viral envelope that is responsible for binding the coronavirus to the host cell receptor and activating the fusion of the viral envelope with the cell membrane (Fischer et al. 1998). The small protein envelope (E), also known as sM (small membrane), is a nonglycosylated transmembrane protein (10 kDa) present in the virion in smaller quantities (Fischer et al. 1998). It plays a pivotal role in the process of coronavirus budding, which occurs in the endoplasmic reticulum and the Golgi apparatus in the intermediate compartment. The matrix (M) protein (25–30 kDa) forms a more abundant glycoprotein membrane that is incorporated by the M–E reaction in the viral particle, while the incorporation of S into the particles is directed by the S–M interaction, being important for the viral maturation of CoV and the site at which the viral particles are assembled (Yuan et al. 2017). The most conserved structural protein in the coronavirus is protein N (45–50 kDa), which is required for the encapsulation of the genomic RNA and its incorporation into the virion. It is also possible that this protein is involved in RNA replication (Risco et al. 1996).

When a host cell is infected, an ORF located 5' from the viral genome is translated into a multiple protein cleaved by viral proteases and then releases other non-structural proteins such as an RNA-dependent polymerase (Rep) and ATPase (helicase) (Hel) (Menachery et al. 2017). These two proteins are involved in the replication of the viral genome and the production of transcripts used in viral protein synthesis. The mechanisms that these genomic RNAs enable are not fully understood, but recent studies have shown that transcriptional regulatory sequences at the 5' end of each gene provide signals that control the sporadic transcription of subgenomic mRNAs (Yang and Leibowitz 2015).

The membrane proteins (proteins S, E, and M) of the virus are fused into the intermediate chamber, while the repeating RNA (positive strand) synthesizes the N protein (nucleocapsid) (Li et al. 2020). The protein–RNA complex binds to protein M located in the membrane of the

endoplasmic reticulum, and forms viral particles when the complex nucleocapsid buds in the membranes of the endoplasmic reticulum. The virus then travels through the Golgi apparatus and eventually leaves the cell, for example, by exocytosis. The virus binding site to the host cell is found at the S protein level (Song et al. 2019).

Efforts to curb infections are being developed around the world. However, many countries are not prepared for this disease, and cannot prevent its transmission or treat it effectively. Various coronavirus vaccines have been developed, including complete attenuated and inactivated vaccines, recombinant S protein vaccines, nucleic acid vaccines, and vector vaccines (Roper and Rehm 2009). The aim of this manuscript is to review existing knowledge about vaccine development and applicability in light of the COVID-19 pandemic, with a special focus on recombinant measles virus expressing the SARS-CoV-2 spike protein.

Types of vaccine under development

There are currently more than 180 candidate vaccines against SARS-CoV-2 in development. The World Health Organization (WHO 2020) has presented a working paper covering many vaccines under implementation.

Inactivated vaccines

Inactivated vaccines are produced by promoting SARS-CoV-2 in cell culture, usually in Vero cells, followed by chemical inactivation of the virus (Gao et al. 2020). Usually, these vaccines are given by intramuscular injection and may contain aluminum hydroxide or other adjuvants. When the main virus is added to the immune system, not only the SARS-CoV-2 antigen but also the matrix, envelope, and nuclear proteins are likely to be targeted by immune responses (Pandey et al. 2020).

Live attenuated vaccines

Live attenuated vaccines are made by creating a genetically weak type of the virus that multiplies to a small degree but does not cause any disease, while triggering immune responses similar to those caused by the actual infection (Talon et al. 2000). Such attenuation can be achieved by modifying the virus under adverse conditions or reasonable alteration of the virus (Broadbent et al. 2016). An important advantage of these vaccines is that they can be administered intranasally, the main entry route for the virus, and thus stimulate mucosal immune responses that can protect the upper respiratory tract. However, the drawbacks of these vaccines include safety concerns and the need for virus modification (Yang

et al. 2020). An inactivated vaccine candidate against the SARS-CoV-2 virus (PiCoVacc) is currently under clinical trial (Risson 2020).

Recombinant protein vaccines

Recombinant protein vaccines can be divided into those based on the recombinant spike protein, Recombinant Receptor-Binding Domain (RBD), and virus-like particles (Chen et al. 2020). The advantage of these vaccines is that they can be produced without dealing with the live virus. However, they also have drawbacks. Indeed, the spike protein is relatively difficult to express, which will probably affect output yields and the number of doses that can be made (Amanat et al. 2020). RBD is easier to express, but when expressed alone is a relatively small protein, and while strong neutralizing antibodies can bind to RBD, other equivalent epitopes present on the full-length spike are absent. This may mean that RBD-based vaccines are more likely to be susceptible to antigen drift compared with those containing the full-length spike protein. Several candidate recombinant protein vaccines against SARS-CoV-2 are currently in preclinical studies, and some spike protein- and RBD-based vaccines have entered clinical trials [Middle East respiratory syndrome (MERS9-CoV vaccine)] (Jain et al. 2020).

Replication-incompetent vectors

A large group of vaccines based on replication-incompetent vectors are under development. Typically, these vaccines rely on another virus that is designed to express the spike protein but prevented from reproducing in vivo by deleting parts of its genome (Mercado et al. 2020). Most such vectors are distributed intramuscularly, entering the vaccinated person's cells and then expressing the spike protein, to which the host immune system responds. Their disadvantage is that some of these vectors may be affected and partially neutralized by preexisting vector immunity (Zhu et al. 2020a, b). This can be circumvented by using vector species that are rare in humans (Mercado et al. 2020) or derived from animal viruses (Folegatti et al. 2020), or viruses that do not induce any immunity per se. Various SARS-CoV-2 vaccines based on replication-incompetent vectors are under clinical development: results for ChAdOx1 nCoV-19 have been reported from nonhuman primate (NHP) trials and/or clinical studies in humans (an AdV26-based vector) (Mercado et al. 2020), and by CanSino (AdV5) (Zhu et al. 2020a, b); additionally, a candidate from Gamaleya Research Institute (Ad5/Ad26) (Logunov et al. 2020) is in phase III clinical trials, and another from ReiThera (Gorilla AdV) is in phase I trials (Forni and Mantovani 2021).

Replication-competent vectors

Replication-competent vectors are derived from an attenuated virus or vaccine strains that are designed to express a mutated gene, in this case for the spike protein. Animal viruses that do not reproduce successfully and do not cause human infection are often used. This approach results in the induction of more stringent immunity, as the vector circulates in the vaccinated person to some degree, at times producing a strong innate immune response. There are currently only two vectors capable of reproduction in phase I clinical trials: an engineered measles vaccine strain developed by the Pasteur and Themis Institute, and a vector based on influenza viruses being developed by Beijing Wentai Biological Pharmacy (Li et al. 2021). However, several other vectors including vesicular stomatitis virus (VSV) (Case et al. 2020) and Newcastle disease virus (NDV) (Rohaim and Munir 2020) are currently under development. NDV-based vectors are of interest because this virus grows to a high egg titer level and the vectors can be generated using the global influenza virus vaccine pipeline (Sun et al. 2020). Unlike measles and VSV vectors, this vector may also be safe enough for intranasal administration, which could induce mucosal immunity.

Inactivated virus vectors

All candidate SARS-CoV-2 vaccines currently under development are based on viral vectors that present the spike protein on their surface but are then inactivated before use (Dong et al. 2020). The advantage of this technique is that, with the exception of in an immunocompromised host, the vector is improved by inactivation so that it does not reproduce. The amount of antigen introduced into the immune system cannot be easily controlled when using ordinary viral vectors, but can be standardized easily for inactivated vector vaccines, as is the case for inactivated or recombinant protein vaccines. Examples of inactivated virus vectors include NDV-based vaccines that present the spike protein on their surface, which can also be produced using a similar route to influenza virus vaccines as well as rabies vectors (Sun et al. 2020). These technologies are currently in the preclinical stage.

DNA vaccines

DNA vaccines are based on plasmid DNA that can be widely produced in bacteria. These plasmids usually contain mammalian expression stimuli and a gene encoding the spike protein, which is expressed in the vaccinated individual upon delivery (Silveira et al. 2021). The major advantage of these techniques is the possibility of large-scale production of *Escherichia coli*, as well as the high stability of the plasmid DNA. However, DNA vaccines often show low immunity,

and they must be administered via delivery devices, such as electrical appliances, to make them effective, which limits their use. Four different DNA vaccine candidates against SARS-CoV-2 are currently in phase I/II clinical trials (Krammer 2020).

RNA vaccines

Finally, RNA vaccines are a relatively recent development. Similar to DNA vaccines, instead of the antigen itself, the genetic material is added to the antigen, which is then expressed in the cells of the vaccinated individual. It is possible to use either mRNA (with modifications) or self-replicating RNA. For mRNA, higher doses of self-replicating RNA are required (Vogel et al. 2018), while RNA is usually delivered via lipid nanoparticles (LNPs). In recent years, RNA vaccines have shown great promise, and many of them are under development, including for the Zika virus and cytomegalovirus (CMV). Different RNA vaccine candidates against SARS-CoV-2 are currently in various phases of clinical trials, e.g., those of the National Institute of Allergy and Infectious Diseases (phase I), BioNTech RNA Pharmaceuticals GmbH (phase II), and Pfizer and ModernaTX, Inc (phase III) (Chakraborty et al. 2021). The advantages of this technology are that the vaccine can be developed entirely in the laboratory. Unfortunately, this vaccine candidate is very unstable, limiting its potential (Chakraborty et al. 2021).

Perspective vaccine strategies: recombinant measles virus expressing the SARS-CoV-2 spicule protein (S)

The vector-based immunization agent platform offers new opportunities as a replicable but safe infectious agent vector. The principle of using MV is based on the following arguments: (1) MV is one of the safest and most effective human vaccines, resulting in lifelong immunity against infection with just one injection (Lorin et al. 2004); (2) Its production could be increased simply and at low cost, which is very important for developing countries where SARS-CoV-2 is endemic; (3) Protection resulting from MV vectors stimulates each mechanism in the body as well as cellular responses to transgenes (Stebbing et al. 2012); (4) The MV arrangement can integrate up to 6 kb into additional transcriptional modules, allowing the expression of multiple primary infection antigens (Ramsauer et al. 2015); (5) Clinical trials and phase II clinical trials using a recombinant MV immunization agent (rMV) expressing Chikungunya virus-like particles have revealed high immunogenicity after immunization, and unlike vector platforms based on non-replicating infectious agents, there is no preexisting immunomodulatory effect against the infectious vector (Reisinger

et al. 2018). The recombinant COVID-19 vaccination agent can thus be incorporated simply into vaccination schedules.

Highly successful vaccines have been developed using live attenuated RNA viruses. MV vaccines cause prolonged human immunity after a single injection (Hilleman 2003). The resulting immunity is highly robust and depends on the induction of an antibody response and a cellular response via CD4 and CD8. The MV genome is very stable, and no side effects of vaccine have been observed (Schneider et al. 2000). MV belongs to the Paramyxoviridae genus of the *Morbiliviruses* family; it is an enveloped virus whose genome is a single-stranded negative-polarity RNA (16 kb), and its cytoplasmic reproduction period excludes any possibility of incorporation into the host genome (Johnston et al. 1999). Hence, MV is one of the commonest and safest live vaccines used in humans. Frédéric Tangy's team recently developed an expression vector based on the Schwarz strain of MV, which is the safest and most commonly used dilute strain in humans. This vaccine strain can be separated from an infectious molecular clone, thus preserving its immunity in both primates and infectious mice. It constitutes a vector for the expression of heterozygous sequences, following the introduction of additional transcription units (Combret et al. 2003).

In addition, recombinant MV Schwarz expressing the envelope glycoprotein of West Nile virus (WNV) induces an effective, long-acting antibody response that protects the mouse from fatal WNV infection. All these characteristics of the attenuated Schwarz strain of the measles virus make it a very promising candidate vector for building new live recombinant vaccines (Desprès et al. 2005).

The aim of using recombinant measles virus (MV) is to evaluate the expression of various SARS-CoV-2 antigens to build new candidate SARS vaccines. Attention should be paid to the SARS-CoV-2 spicule (S) protein, which allows induction of antibodies that neutralize SARS-CoV-2 infection after gene immunization in animals, as well as the soluble and secreted form of this protein, Ssol polypeptide. Ssol polypeptide contains antigens similar to the S protein and allows the induction of high titers of neutralizing antibodies against SARS-CoV-2 after injection into mouse in the form of a protein adjuvant in aluminum hydroxide. The various variants of the S gene in the cDNA of the MV Schwarz strain are inserted in the form of a transcription unit between the P (phosphoprotein) and M (template) genes. After isolating the recombinant viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol and verifying their ability to express SARS-CoV-2 S antigen, their ability to induce an immune response should be tested in mice to achieve protection against SARS-CoV-2.

The spike (S) antigen is a promising candidate and has been studied for the development of SARS-CoV-2 vaccines due to its primary role in recognizing receptors as well as the

binding and entry of the virus into the cell. This is the main target of inactivating antibodies in humans (He et al. 2005) and animal models (Tripp et al. 2005).

Moreover, through research on SARS-CoV-2 and associated MERS-CoV vaccines, it is well known that the S protein on the surface of the virus is a suitable target for a vaccine. In SARS-CoVs, this protein interacts with angiotensin-converting enzyme 2 (ACE2) receptors, and spike-targeting antibodies will interfere with this binding, thereby neutralizing the virus (Lan et al. 2020). The structure of the SARS-CoV-2 S protein was resolved with high precision in record time, leading to an understanding of this vaccine target (Wrapp et al. 2020). The target antigen is thus ready for inclusion in advanced vaccine platforms, although the genomic makeup could vary and exhibit mutation from one country to another. The S protein sequence (493 bp)

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1  ttcaagactc actttcttcc acagcaagtg cacttggaac acttcaagat gtgggtcaacc
61  aaaaatgcaca agctttaaac acgcttgta aacaacttag ctccaatttt ggtgcaattt
121 caagtgtttt aaatgatata ctttcacgtc ttgacaaagt tgaggctgaa gtgcaaatg
181 atagggtgat cacaggcaga cttcaagtt tgacagata tggactcaa caattaatta
241 gagctgcaga aatcagagct tctgctaac ttgctgtac taaaatgtca gagtgtgtac
301 ttggacaate aaaaagagtt gattttgtg gaaagggtc tcatcttatg tctctcctc
361 agtcagcacc tcattggtga gtctcttgc atgtgactta tgcctgtca caagaaaaga
421 acttcacaac tgctctgtcc attgtcatg atggaaaagc acacttctc cgtgaagggtg
481 tctttgtttc aaa

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Fig. 1 Membrane protein S genomic sequence of COVID-19 in two Tunisian patients (El Moussi et al. 2020)

in Tunisia has already been described (Fig. 1) (El Moussi et al. 2020).

Construction of recombinant viruses

The pTM-MVSchw-ATU2 plasmid (Fig. 2) contains infectious cDNA corresponding to the Schwarz MV strain antigen as an additional transcription unit (ATU), inserted between the P (phosphoprotein) and M (matrix) genes (Combret et al. 2003). The recombinant measles virus genomes MVSchw2-SARS-S and MVSchw2-SARS-Ssol will be generated by inserting ORFs of the S protein and Ssol polypeptide into the additional transcription unit of the MVSchw-ATU2 vector (Fig. 3).

Recombinant MVSchw2-SARS-S and MVSchw2-SARS-Ssol cause expression of the S protein and Ssol polypeptide, respectively, at levels similar to those observed 8 h after SARS-CoV-2 infection. Expression of these polypeptides will be stable after three cycles of recombinant viruses in cell culture. These results demonstrate that recombinant MV could well suited to carrying transgenes and enable expression of the SARS glycoprotein in its membrane (S) or soluble form (Ssol). It is expected that Ssol polypeptide will be secreted from MVSchw2-infected cells as SARS-Ssol when the same polypeptide is expressed in mammalian cells after transient transfection of the corresponding sequences.

Many concerns about viral evolution persist regarding the global spread of the COVID-19 pandemic in 2020. When viral mutation occurs naturally or by accident, it is always difficult to distinguish. It is also difficult to predict whether the outcome of an outbreak or pandemic will change after a single mutation. Korber's recent research provides compelling data indicated that an amino acid

Fig. 2 pTM-MVSchw-ATU2

Fig. 3 Construction of pTM-MV Schw2-SARS-S and pTM-MV Schw2-SARS-Ssol

change in the virus spike protein, D614G, occurred early in the pandemic, and that viruses containing G614 are now prevalent in many places around the world (Korber et al. 2020).

Transmission is the key process for viruses such as SARS-CoV-2, if they do not enter another host which ends the strain. Korber et al. (2020) hypothesized that the rapid spread of G614 occurred because it was more contagious than D614. The authors, in support of their hypothesis, provided evidence that clinical samples from G614 infection contained a higher level of viral RNA, and the results of in vitro studies produced higher titers of pseudoviruses, which now seems to be supported by other results (Hu et al. 2020; Wagner et al. 2020). However, this evidence does not indicate that viruses including G614 are more contagious or more transmissible than those with D614. Because of this, many concerns remain about the potential, if any, impacts of D614G on the COVID-19 pandemic. It will also be important to verify through more detailed clinical studies whether these mutations really do not have the potential to cause more serious disease. Finally, although these mutations are relatively rare in coronaviruses, this study highlights the need to continue monitoring the progress of the epidemic, particularly to detect immune-escape mutations that may be important for vaccine development strategies.

On the other hand, according to Voskarides, since people exposed to severe infection die and high-mortality virus strains disappear with these people, the disease may become milder in the coming years or decades (Voskarides 2020). According to its development from 2003 to 2021, the SARS-CoV-2 virus has exhibited continuous change, meaning that, if severe infection ends with death, mutation can still produce other deadly strains over the years. The best solution is thus to develop a successful vaccine to eradicate this virus.

Concluding remarks

In the wake of the 2003 SARS pandemic, there was renewed global interest in continuing research and investment to develop effective and safe vaccines against coronaviruses.

SARS-CoV-2 has spread dramatically in several countries, causing serious illness and person-to-person transmission, requiring immediate intervention and care. Biologists must develop drug application strategies to prevent and control transmission.

MV is an effective class of curative agents to counteract this virus. The efficacy of recombinant viruses in treating many diseases is well known, and this strategy will provide safe and successful vaccines that do not recombine or fuse the MV genetic material, as such vaccine strains to defend against SARS virus will be genetically stable and not survive or spread.

MV has demonstrated its potent ability to enhance cellular and humoral immunity against several antigens and defend against experimental challenges in both mice and primates (Brandler et al. 2007).

We propose herein a strategy for developing MV Schw2-SARS-S and recombinant MV Schw2-SARS-Ssol viruses that can induce neutralizing antibodies against SARS-CoV-2. Such candidate vaccines merit evaluation in a more appropriate model than NHP. In addition, various studies have highlighted the need to stimulate large-scale neutralizing antibodies capable of protecting against heterologous viral variants that could occur during further emergencies (Bolles et al. 2011).

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