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Review article

Vaccination strategies to combat novel corona virus SARS-CoV-2

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ABSTRACT

The 2019-novel coronavirus disease (COVID-19) is caused by SARS-CoV-2 is transmitted from human to human has recently reported in China. Now COVID-19 has been spread all over the world and declared epidemics by WHO. It has caused a Public Health Emergency of International Concern. The elderly and people with underlying diseases are susceptible to infection and prone to serious outcomes, which may be associated with acute respiratory distress syndrome (ARDS) and cytokine storm. Due to the rapid increase of SARS-CoV-2 infections and unavailability of antiviral therapeutic agents, developing an effective SAR-CoV-2 vaccine is urgently required. SARS-CoV-2 which is genetically similar to SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) is an enveloped, single and positive-stranded RNA virus with a genome comprising 29,891 nucleotides, which encode the 12 putative open reading frames responsible for the synthesis of viral structural and non-structural proteins which are very similar to SARS-CoV and MERS-CoV proteins. In this review we have summarized various vaccine candidates i.e., nucleotide, subunit and vector based as well as attenuated and inactivated forms, which have already been demonstrated their prophylactic efficacy against MERS-CoV and SARS-CoV, so these candidates could be used as a potential tool for the development of a safe and effective vaccine against SARS-CoV-2.

1. Introduction

In December 2019, a large number of patients with the symptoms similar to pneumonia with unknown cause were detected from the city of Wuhan in Hubei province in China [1,2]. Reports say that most of the original cases had close contact with a local animal and seafood market of Wuhan, China [3,4]. Further, the detailed sequence analysis of the sample taken from lower respiratory tract of a patient revealed the existence of, a novel coronavirus, later named as SARS-CoV-2 that was never been reported earlier in humans [5]. Infections with SARS-CoV-2 are now widespread around the globe and are increasing exponentially every day. A total of 6 million cases with SARS-CoV-2 infections have been confirmed up to May 29th, 2020, and 360,000 people have died across the world.

Coronaviruses (CoVs) are the largest group of viruses belonging to family Coronaviridae and the order Nidovirales [6,7]. They are either pleomorphic or spherical in shape with a diameter of 125 nm [8,9]. Further they are characterized by club shaped spike projections on its surface. Transmission of COVID-19 is mainly caused by respiratory droplets, direct human to human contact and fecal to oral transmission might be also associated [10]. COVID-19 prominently affect the respiratory tract (both lower and upper respiratory tract), with the initial symptoms of common cold, fever, dry cough, fatigue, nasal congestion, sore throat and diarrhea to severe pneumonia, difficulty in breathing and ends with the patient death [11]. The incubation period of the disease is 14 days and the time from onset of symptom to developing pneumonia is 4 days [10]. The reservoir hosts of the disease are bats and Himalayan palm civets [12] but the scientists in Guangzhou have reported coronavirus from Pangolin the ant eating mammal with 99% homology with a receptor binding domain matching to that of SARS-CoV-2. However, this is not confirmed yet [13]. These are the enveloped viruses that contain, segmented, positive sense, SS-RNA genome of approximately 30 kb which is the largest among all the RNA viruses [14]. The genetic material of CoV is highly prone to frequent recombination process that results into the formation of new strains with altered virulence [15]. There are four relatively 'benign' strains of human coronaviruses (hCoVs) 229E, NL63, OC43, and HKU1 causing mild respiratory flu like seasonal illnesses, however three strains (SARS-CoV, MERS-CoV, and SARS-CoV-2) are reported to be extremely pathogenic. It is thought that SARS-CoV-2 might be the next series of the previous two outbreaks (SARS-CoV, MERS-CoV) as the CoVs are the emerging viruses, which are highly adapted to the changing environment. Further with its genome closely related to SARS-CoV and MERS-CoV and the accumulated clinical and experimental data on these

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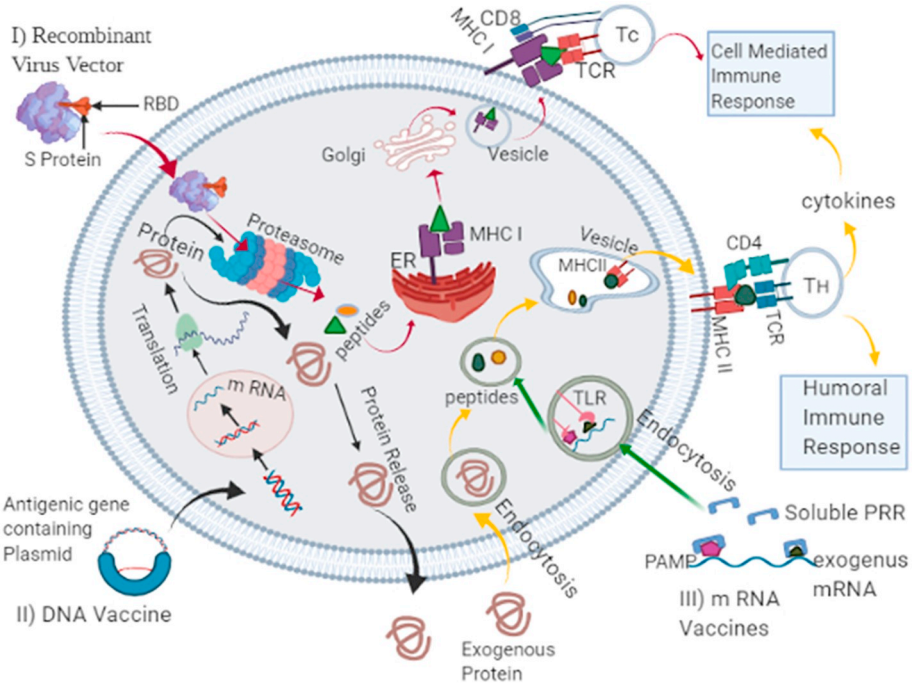

Fig. 1. Mechanism of action of various vaccine candidates: I) Recombinant Virus Vector act as an endogenous antigen, thus after processing in the proteasome, they are presented by MHC I to the CD8+ Tc cells leading to Cell Mediated Immune (CMI) response; II) DNA Vaccine are transcribed and translated in the host cell, the protein synthesized then moves to MHC class I pathway or the protein would be released outside the host cell where it act as an exogenous antigen and are presented by MHC II to the CD4+ TH cells leading to humoral immune response, also release of some cytokines by TH cells leads to CMI response as well; III) mRNA vaccines exposed PAMP (Pathogen Associated Molecular Pattern)are recognized by the soluble PRR (Pathogen Recognition Receptor) endocytosed and lead to MHC class II pathway, Thus eliciting the humoral immune response.

previous viruses we can precisely predict the possible vaccine candidates to prevent against CoV infection in the future [16]. Currently there is no specific antiviral drug against SARS-CoV-2, finding a vaccine for this virus therefore remains a high priority [17]. In this review we aim to discuss about different approaches for developing an effective vaccine against COVID-19.

2. Mechanism of action of various vaccine candidates

The most effective licensed vaccines elicit long-term antigen-specific antibody responses by plasma cells in addition to the development of persisting T cell and B cell memory. In case of SARS-CoV infection both humoral and cellular immune responses are crucial for the clearance of infection (Fig. 1). Recombinant virus vectors work in a similar manner like an endogenous pathogen, by expressing axenic target protein in cytoplasm of the host cell. After, processing of such endogenous antigen, MHC class 1 molecules present them to CD8+ T-lymphocytes, which causes production of T-cytotoxic cells. This pathway, leads to establishment of cell-mediated immunity, which is crucial in getting rid of virus infected cells. Sub unit vaccine candidate particularly RBD of S protein of SARS-CoV contains major antigenic determinants that can induce neutralizing antibodies [18]. The SARS-CoV S protein can also induce CD8+ T-cell responses. The RBD of S protein contains multiple conformation-dependent epitopes and is the main domain that induces neutralizing antibody and T-cell immune responses against SARS-CoV infection [19,20] making it an important target for vaccine development. The approaches for developing RBD-based vaccines against SARS-CoV have provided useful information for designing safe and effective vaccines against SARS-CoV 2 since RBDs of SARS-CoV 2 also contain similar epitopes. Similarly Adenoviral vectors are able to induce potent antibody as well as T cell responses with variations in the immune response depending on the serotype employed [21]. Replication-deficient Ad5, one of the most widely used adenoviral vectors, is able to induce exceptionally potent CD8+ T cell as well as antibody responses [22]. Furthermore DNA vaccination is also able to elicit both humoral and cellular immune responses, through activation of CD8+ cytotoxic and CD4+ helper T cells, respectively, Upon entry in the cell, DNA vaccines are sensed by a variety of innate immune receptors i.e. STING/TBK1/IRF3 pathways and the AIM2 inflammasome and many other factors are involved in DNA vaccine mode of action [23] but the exact mechanism of action is yet to be evaluated. However, immunization with S protein encoding DNA vaccine elicited protective immunity against SARS-CoV infection in a mouse model by inducing T cell and neutralizing antibody responses [24].

Another nucleotide based vaccine i.e. Exogenous mRNA is also immunostimulatory, as it is recognized by a variety of cell surface, endosomal and cytosolic innate immune receptors. Mammalian cells can sense foreign RNA via Pattern recognition receptors (PRRs) such as TLR3, TLR7 and TLR8 located in the endosomes and RIG-I, MDA-5 and PKR located in the cytoplasm as well as NLRP3 and NOD2 [25]. Activation of the PRRs by mRNA vaccines results in a robust innate immune response including production of chemokines and cytokines such as IL-12 and TNF at the inoculation site [26], which are innate factors crucial for the induction of an effective adaptive immune response against the encoded antigen. ID immunization with mRNA vaccines upregulates the expression of chemokines including the CXCR3-ligands CXCL9, CXCL10, and CXCL11, that recruit innate immune cells such as DCs and macrophages, to the site of injection [26]. The mRNA vaccines can also induce an immunological repertoire associated with the generation of high magnitude long-lived antibodies [27].

3. Vaccination strategies

Viral zoonosis had resulted into many disease epidemics in recent years and emergence of new strains from their zoonotic hosts makes them very difficult to predict [28,29]. Earlier, CoV were thought as weak pathogen for humans causing mild flu-like illness but with consistent outbreaks like SARS in 2002, MERS in 2012 and now COVID-19 their pathogenicity is very well established globally [30,31]. Such repeated transmission leading to global economy losses makes CoV vaccines highly desirable, as presently there are no antiviral drugs available against CoV. Various areas explored for the search of an ideal vaccine against SARS-CoV, includes inactivated virus vaccines, recombinant viral vaccines, subunit vaccine, DNA vaccines and attenuated vaccines [32]. The antigenic targets selection and vaccination strategies are probably based on SARS-CoV and MERS-CoV vaccine studies, summarized in Table 1.

Table 1
The possible strengths and weaknesses of different types of vaccination strategies against SARS-CoV and MERS-CoV.

Vaccines strategies	Vaccine candidates	Phase	Advantages	Disadvantages	Reference
Nucleotide based	**DNA vaccines**	Phase I,II (NCT03721718)	Simple, stable, safe and easy to produce, cost-effective; induce neutralizing antibodies; human monoclonal antibodies and cytotoxic T-lymphocyte response	Lower immunogenicity as compared to inactivated and live-attenuated virus vaccines; require any additional administration device	[23,36,39,47,48]
	S, M and N genes **mRNA vaccines**	Phase I (mRNA-1273) and preclinical (BNT162)	Easy to design; manufacturing is a comparably short, simple, rapid and cost-effective; avoid the risk of host genomic integration; safer than inactivated or protein based vaccines as they are free from the risk of protein contamination or the injected virus to become active; induce both humoral and cellular immune responses	Unprotected naked mRNA alone is not very stable thus requires highly efficient carriers to stabilize and pack the mRNA into an injectable form	[26,27,52]
	mRNA-1273 and BNT162 encoding S protein				
Subunit vaccine	Spike glycoproteins (S), membrane proteins (M), nucleoproteins (N)	Preclinical	High safety profile; less prone to generate the side effects at the site of inoculation; involving pure antigens that only includes synthetic peptides or recombinant proteins that expresses only particular fragments of immunogen; consistent production; eliciting T and B cell mediated immunity	Require appropriate adjuvant; cost-effectiveness may vary; lower immunogenicity	[24,49,63,67]
Recombinant vector vaccines	Coronavirus proteins/glycoproteins expressed by attenuated adenovirus/ poxvirus/Newcastle disease virus	Phase I (NCT03399578, NCT03615911)	Quickly constructed; Efficient in inducing immune response; ease of administration and production; elicit long-termed cell mediated and humoral immunity	Restricted cloning ability, limited host range and pre-existent immunity; route of administration that manifest different immune response; possible Th2-bias	[83,84,86,93,100]
Attenuated vaccines	Gene deletion of various essential genes (S,N,E genes), nonstructural proteins (nsp) encoding genes	Preclinical	Persists for longer period of time, showcases the entire viral antigens complement to the host immune system, efficient in generating a strong cytotoxic T-cell response	Risk of reversion to its virulent form; requirement of cold chain; not appropriate for infants, immunocompromised or elderly individuals	[106,110]
Inactivated virus vaccines	Inactivated or whole killed virus (WKV)	Preclinical	Rapid development; efficient in inducing immunity and protection against virus infection; efficiently neutralizes induction of antibody; can be formulated with different adjuvant	Hypersensitivity Th2-bias	[89,90,125]

3.1. Nucleic acid vaccines

Antigen that encodes either plasmid DNA or RNA i.e. mRNA or viral replicon, are used in the nucleic acid based approaches. After being taken and expressed by a cell, these antigens, which are encoded by nucleic acid, induce antibody and cell -mediated response as well. Owing to the simplicity in the alteration of antigen they permit, both the approaches are tremendously adaptable. Antigen production in the target cells suggests the benefit of imitating synthesis of protein throughout infectivity. Prominently, they allow any preference antigen delivery, despite of the fact that it was either isolated from bacteria, virus or any parasite thereby permitting development of vaccine against broad pathogen group. Further vaccine characteristics are not dependent on encoded proteins so, there is no need to set up new production, purification, validation methods and manufacturing services for production of nucleic acid based vaccines in large scale.

3.1.1. DNA vaccines

DNA vaccination confers an attractive approach for developing a SARS vaccine due to their simplicity, stability, safety and easy production ability as compared to other conventional approach. In the field of DNA vaccination substantial progress have been recorded in the past decades with the production of HIV-1 vaccines (the first DNA vaccine) demonstrated in human, which depicts the safety and protective efficacy of the vaccine [33]. Besides this various other DNA vaccines including AIDS [34], malaria and influenza [35] are also under human trial.

DNA vaccine made up of plasmid DNA encoding protein from pathogens have been demonstrated for inducing both T and B cell responses, after showing the similar effects of live viruses, that antigenic proteins are produced endogenously and presented efficiently by MHC class I, thus help in inducing CD8 + T-cell responses [36]. Further as the DNA vaccines are simple, stable, safe and can be easily produced it can be possible option to the use of live vaccines [36]. There are numbers of SARS-CoV proteins viz. S [24,28,37–40], M [41] and N [42,43] proteins reported to induce cellular and humoral responses have been utilized as DNA vaccine candidates [40]. In a study DNA vaccine associated expression of S protein have generated both T-cell and neutralizing antibody responses, and lowered the replication of SARS-CoV in the respiratory tract this manifested that protective efficacy to the S gene in mice was antibody mediated not T-cell dependent [24]. Further in a study S plasmid improved the gene expression of spike (S) protein by heterologous viral RNA export elements and protection in mouse model [39], but their role have not been recorded in case of other in vivo models. Vaccination of mice with multi-epitope DNA vaccine has elicited specific antibody response to two candidate epitopes, S(437–459) and M(1 − 20) which reduced the virulence of SARS-CoV in-vitro, but there was no report on the protective efficacy [41]. In another study mice Immunized with N-DNA vaccine induced N-specific antibody response and Tc-cell activity [42,44], moreover a strong delayed-type hypersensitivity (DTH) and CD8(+) CTL responses to N protein was observed [44]. In an another study of N-DNA vaccination, expression of N protein and its linkage to LAMP for better presentation of MHC II has amplified memory response [45]. Moreover vaccination of C57BL/6 mice with naked CRT/N DNA for improved MHC class I presentation not only induced N-specific humoral and cellular immunity but also considerably reduced the titer of challenging vaccinia virus against the cells expressing N protein [43]. In addition to other animal models, an N-DNA vaccine candidate has also been evaluated in HLA-transgenic mice that induced a specific CD8 + T-cell response [46]. Besides the expression of S and N proteins, M protein have also been reported to be expressed in mice and found to induce neutralizing antibodies, human monoclonal antibodies and cytotoxic T-lymphocyte response [47]. In a comprehensive study of DNA vaccine using different gene fragments (S-, M- and N), M DNA vaccine was reported with the highest cellular immunity in mice [40]. Although DNA vaccines works efficiently

during preclinical stages, but their efficacy in field conditions are quite unsatisfactory and have lower immunogenicity [48], as compared to other vaccines such as inactivated and live-attenuated virus vaccines and need further improvement regarding the efficacy. To confront this problem different prime–boost approaches such as heterologous prime or boost with proteins, inactivated viral vaccine candidates or viral vectors have been constructed [28,41,49–51].

3.1.2. m-RNA vaccines

There are various practices mostly the traditional one that has been approached to make a vaccine, but these methods on an average takes, 16 years as in the case from human papilloma virus, to rotavirus. The development of a protein-based vaccine requires growth of the viruses and its protein purification, which is a lengthy process and may take several years.

To make an effective and new generation vaccine against this fast spreading virus, instead of protein, mRNA vaccination strategy can be a better approach [52]. The mRNA vaccine manifests the immune responses similar to natural infection and provides a short synthetic viral mRNA, which is then used by the host to produce the antigen proteins. Since the mRNA used in vaccine after injected can't integrate into the host's genome, they are safe to use. Furthermore, they are safer than inactivated or protein based vaccines because they are free from the risk of protein contamination or the injected virus to become active. The huge benefit of mRNA vaccine is that it can circumvent the hassle associated with the purification of viral proteins that saves the time from months to years used in standardization and scaling up the mass production [52]. So far there is no approved RNA vaccine, but are under clinical trials. The National Institute of Allergy and Infectious Disease (NIAID), USA in collaboration with Moderna have initiated a research to develop an mRNA vaccine called mRNA-1273 [53] which produces a stable form of the SARS-CoV-2 spike protein (S). NIAID has registered a phase 1 clinical trial of the vaccine candidates on 45 healthy adults completed on 19th March 2020 in Seattle [54,55], while the other clinical trials of phase 1 mRNA vaccines (mRNA-1273) and another mRNA-based vaccine candidate BNT162, currently in preclinical testing are expected to start in April 2020 [53,56]. However, the proposal of mRNA based vaccine development is not new. Since extracellular ribonucleases is everywhere in the cytoplasm, so the unprotected naked mRNA alone is not very stable to be used as a prophylactic vaccine. Due to the presence of strong net negative charge and hydrophilic nature, the RNA cannot be taken up easily by the cells during in vivo condition. Thus it requires highly efficient carriers such as new generations of liquid nano particles (LNP) to stabilize and pack the mRNA into an injectable form which is also done in the case of the mRNA-1273 vaccine being developed today.

Thus DNA and mRNA vaccines that are easier to design and proceed into clinical trials very quickly are excellent target for development of vaccines against SARS-CoV 2 and other related outbreaks in future.

3.2. Subunit vaccine

Considering safety efficacy, subunit vaccines involving pure antigens are said to be advantageous, as the constituent only includes synthetic peptides or recombinant proteins that expresses only particular fragments of immunogen, excluding the participation of contagious viruses. In addition to this, subunit vaccines are less prone to generate the side effects at the site of inoculation. Due to stable conditions and definite fragments of pathogen, subunit vaccines achieve reliable production. Due to all these qualities of subunit vaccines are helpful in making it an interesting candidate for vaccine. A number of structural proteins comprising spike (S), envelope (E), membrane (M), and nucleocapsid (N) are expressed by SARS-CoV that may act as antigens to activate neutralizing antibodies and generate defensive response.

3.2.1. S protein subunit and RBD

Spike protein (S) of virus performs significant part in entering the host [57]. The CoV-RNA genome is allowed to go inside the cells of host through the early interactions of S1 domain to its receptor in the host i.e. PP 4 in MERS-CoV and ACE2 in SARS-CoV, followed by the viral and host membrane fusion facilitated by the S2 segment. To develop vaccines based on CoV subunit, S proteins are the preferred candidate, because S proteins have sites for binding of receptor as well as for membrane fusion; vaccines based on S protein are likely to activate antibodies that prevent the binding of virus and later fusion of membranes as well thereby counteracting infection of virus [58]. S glycoprotein of SARS-CoV which is shown to bind the cellular receptor angiotensin-converting enzyme 2 (ACE2), is an interesting candidate to target for both vaccine and therapeutic improvement [32,59]. This practice is robustly encouraged by the discovery that illustrates the infectivity by SARS-CoV is powerfully counteracted by human mAb that binds to S protein through N-terminal and thereby prevents binding of receptor by hindering the formation of syncytia [60]. On the other hand, few epitopes that are not neutralizing are also present in the immmuno-predominant sites of the S protein of SARS-CoV [61] that could elicit the immune responses which might be harmful [62]. For that reason, the identification and elimination of such epitopes in S protein is obligatory. Moreover, the S protein have been reported to produce serum neutralizing antibodies and protected African green monkeys and mice challenged with SARS-CoV [24,63]. S-ectodomain proteins of full length merged with/without S or S-foldon i.e. fold on trimeric motif, may induce precise antibody response and neutralizing antibody thus causing protection of the vaccinated mice to SARS-CoV infection [64]. Besides this, S protein associated with SARS-CoV is also shown to be responsible for eliciting responses by T-cell (CD4+ and CD8+) [49].

RBD is a segment in the centre of S1 subunit of S protein which is ~193 AA residues and binds to receptors present in the target cells. It has been illustrated that antisera of SARS infected person and that of animals inoculated with inactivated SARS-CoV, effectively responded with RBD [65,66]. A lot of the neutralizing antibodies were removed of these antisera by RBD through antibodies absorption, while the antibodies specific to RBD, having effective neutralizing action were obtained from these antisera [66]. It has also been represented that the RBD vaccinated mice and rabbits produces counteracting antibodies in high number against the SARS-CoV having 50% neutralizing titers at a > 1:10,000 serum dilution [67]. As compared to S protein of full length, high number of neutralizing antibodies were induced with RBD [24,67,68] because in contrast to S protein with full length, no immunodominant regions are present in RBD that elicits antibodies that are not neutralizing. To prevent the infection from SARS-CoV, with different genotypes RBD containing recombinant proteins or vectors that encodes RBD could be used. Thus, the generation of antibodies targeting the RBD subunit of SARS-CoV-2 would be an important preventive and treatment strategy that can be tested further in suitable models before clinical trials.

3.2.2. M and N protein subunits

Besides the above mentioned proteins, expression of various other proteins have also been reported on the virion surface with the production of antibodies in SARS patients serum [69], further these proteins can also be evaluated to check the protective efficacy against SARS-CoV. For instance, counteracting action of the antibodies toward M proteins has also been reported [70]. Membrane or M protein plays an essential role in upholding the configuration of viral envelope [71] and M protein carries out this by making interactions with different CoV proteins [72], incorporating Golgi complex into novel virions [71], and stabilizing proteins of the nucleocapsid [71]. Investigations on animal CoV vaccines have reported that the Nucleocapsid protein (N) of CoV could serve as additional target for the development of vaccine [73]. Even though the antibodies to N proteins of CoV does not have

mechanism to neutralize the virus, but generation of CoV-restricted CD8+ T cells has been reported [74–76], consequently providing protection subsequent to infection in animals [77] therefore, this suggest that in vivo defense could be offered by the protein by eliciting cell-mediated response [74,78]. Research have shown that precise IgG antibodies, as well as subtypes IgG1 and IgG2a were induced when Freund's adjuvant was conjugated with SARS-CoV N protein that was plant expressed while, conjugating adjutants like cysteine-phosphate-guanine (CpG) and Montanide ISA-51 with N protein that was *E. coli*-expressed, results in induction of definite IgG antibodies toward a Th1 (IgG2a)-type response in mice [79,80]. However, the conserved N protein across CoV families implies that it is not a suitable candidate for vaccine development, and the antibodies against the N protein of SARS-CoV-2 do not provide immunity to the infection [81].

3.3. Recombinant vector vaccines

Recombinant vector vaccines can be defined as live, dividing viruses that are genetically manipulated to harbor some additional pathogenic gene, coding for foreign proteins, responsible for eliciting immune response [82]. Recombinant vector vaccines are highly efficient in triggering an immune response as they can infect cells and sustain in the body for quite a long time and can react to antigen-presenting cells directly. Also viral proteins in such vaccines can act like adjuvant [83], thereby enhancing immune response, resulting in generating more antibodies and long-term immunity, and reducing the dose of antigen required. Several attempts have been made in the direction of the development of SARS-coronavirus (SARS-CoV) vaccine for which various viral vectors are genetically engineered to express SARS-CoV proteins in them. Adenovirus and modified vaccinia virus Ankara (MVA) are the two most common viral vectors used in the development of SARS-CoV and MERS-CoV vaccines.

3.3.1. Adenovirus vectored vaccine

Adenovirus based delivery systems have several benefits including their ease of administration by oral or nasal route, their non-pathogenicity in humans [84], particularly for mutants with compromised replication [85]. Defending power of Ad-vectored vaccines against SARS-CoV was first demonstrated in rhesus macaques in 2003 [86]. They were immunized with three Ad5–SARS-CoV Ad-based vector constructs expressing genes for N, S and M proteins. The vaccine induced antibody reaction against spike S1 fragment, production of SARS-CoV neutralizing antibodies and T-cell proliferation against the N protein [86]. In a similar study when Ad vector expressing SARS N- protein was used to vaccinate C57BL/6 strain mice, surprisingly no neutralizing antibodies were produced but SARS-CoV-specific IFN-γ secretion and T-cell production was detected [84]. When 129S6/SvEv strain mice was co- immunized with 2 Ad vector constructs containing genes coding for SARS-CoV S and N proteins by either nasal or muscular route, it suggested that former induced Ig A and effectively checks SARS-CoV reproduction in nasal and alveolar tissues while the later works by proliferating neutralizing antibodies [87]. A number of studies analyzing efficacy of these vaccines have been done in ferrets, being it the only model that resembles most of the symptoms of human patients including fever, transmission by respiratory route, discharge of virus from upper airways and lung injuries [88,89]. When ferrets were primed with adenoviral construct coding S-protein and boosted with a different adenovirus from chimpanzee, a significant reduction in viral load and other clinical symptoms was observed [90]. Targeting that serotype to which humans are naïve is the focal point of recent adenoviral constructs. For this Chimpanzee adenovirus (ChAdOx1) is a promising candidate owing to its lack of persistent immunity in humans [91] and superior safety status. Presently, a potential MERS-CoV vaccine named MERS001 is at clinical trial, having ChAdOx1coding for S-protein of MERS-CoV [92]. But restricted cloning ability, limited host range [93] and pre-existent immunity against adenovirus in significant proportion of human population [83,94] are some of their limitations which makes their animal testing challenging and retards their clinical use as a vector for vaccine designing.

3.3.2. Poxvirus vectored vaccines

The Poxvirus constructs are potent vectors due to better stability, greater insert size, easy production, cytoplasmic gene expression and capacity to elicit long-termed cell mediated and humoral immunity [83]. In a study, replication-compromised poxvirus vector, modified vaccinia Ankara (MVA) strain, coding for SARS-CoV S protein, generate neutralizing antibody response and reduced viral load in the upper airways of mice [63]. MVA constructs expressing entire S protein are potent vaccine target [95–97]. An additional vaccine target is MVA-MERS-S, presently in phase I clinical trial at University Medical Center Hamburg-Eppendorf, Germany [98]. Studies involving relatively conserved N protein expressing MVA in mice generated $CD8^+$ cells [99].

3.3.3. Newcastle disease virus vectored vaccines

Newcastle disease virus (NDV) viral-vector contain MERS-CoV S protein which generated neutralizing antibody response in BALB/c mice and Bactrian camels [100]. Although NDV vaccines elicited vigorous immune response, but also have many limitations namely, persistent immunity against NDV, higher threat of pathogenesis, low viral titer production, and possible carcinogenesis [101].

Since viral vector-based vaccines could be quickly constructed and used without an adjuvant, development of such vaccines might be possible in SARS-CoV-2 because RBD of S-protein has been identified as a neutralizing epitope [102]. So this epitope could be actively pursued for the development of viral vector based vaccines.

3.4. Attenuated vaccines

Historical evidences demonstrate that live attenuated vaccines are the most successful vaccines against intracellular pathogens as they have already proven in case of various bacterial and viral pathogens [103–105]. It is an attractive approach where attenuated live virus confers immune responses similar to natural infection, without posing any risk of infection and provides long lasting protective immunity. Development of a vaccine should follow some stringent criteria where safety, efficacy and reproducibility are of paramount significance. These vaccines are more efficient as they persists for longer period of time, showcases the entire viral antigens complement to the host immune system, deliver antigen to the appropriate compartments of cells and tissues to produce endogenous proteins, efficient in MHC class I presentation and finally help in generating a strong cytotoxic T-cell response. Further it improves the potential of live attenuated vaccines to support antigen-specific effectors and memory immune responses so that a long term protective immunity can be achieved. However, attenuated vaccination is a promising approach in the field of viral infection, but the main difficulty is the reversion of the pathogen to its virulent form as happened in the case of oral vaccine of poliovirus [106]. From the safety point of view, it is often very difficult to get vaccine regulatory approval and using such vaccines without strong evidence is a huge risk. Although this limit for SARS has not yet been met, but some attractive attenuated mutants have been developed. Deletion of E protein encoding gene abrogated the virulence of CoVs, and several studies have explored the potential of recombinant SARS-CoV or MERS-CoV with a mutated E protein [107,108]. Further, vaccination of hamsters with this mutant have shown raised level of serum-neutralizing antibodies and protected from clinical sign and replication of homologous (SARS-CoV, Urbani) and heterologous (GD03) SARS-CoV in the respiratory tract (both the upper and lower respiratory tract) [109]. Thus, deletion of the structural E gene may prove to be the first step in the field of developing a live-attenuated vaccine against SARS-CoV. Moreover, *nsp-1* gene deleted CoV mouse hepatitis virus (MHV) has also been reported with high efficacy thus can also be used for

developing a SARS-attenuated vaccine [110]. Two studies related to gene deletion and attenuation effects suggests that removal of ORFs 3a, 3b, 6, 7a, 7b, 8a, 8b or 9b results in very little and no viral replication during in-vitro and in vivo conditions [108,111]. However the outcomes of the studies conducted so far are not so convincing because it takes longer time to create non-infectious clones of coronavirus due to larger genome size and also requires extensive safety testing [112]. Furthermore, reversion of the pathogen to the virulent form can be protected using gene replacement knockout strategy that can be safer as compared to the lines developed by other means like chemical treatment and irradiation etc.

3.5. Inactivated virus vaccines

Inactivated virus vaccines also known as the WKV (whole Killed Virus) vaccines represent a pathogen whose ability to infect and replicate has been ceased, consequently making it sterile but retaining its ability to act as an immunogen, so that the immune system could still work if such a pathogen is injected into a host. Inactivated vaccines are prepared by neutralizing the pathogen as a whole by chemicals or by heat and radiation. It is thought that inactivated vaccines can be prepared with much less effort which makes them one of the attractive types of vaccines prepared in the market today. These vaccines work by exposing the same epitopes which a virus otherwise would have presented, thus eliciting an immune response. When sera of the infected person were tested, presence of antibodies against minimum eight different proteins suggests the presence of diverse moieties exposed on the membrane of the pathogen [69]. In order to search targets for the protective antibodies further investigations has been done and it is found that additional structural proteins that can be targeted are encoded by (Open Reading Frame) ORFs (3a, 6, 7a and 7b), envelope (E), spike (S), and matrix (M) [49,113–116]. By interpreting these data we can assume that multiple targets are present for the protective antibodies. 3CL, nucleocapsid (N), S, M etc. are the proteins against which the antibodies were induced when a mice was vaccinated with SARS-CoV [117]. Despite many advantages there are some limitations for the development of inactivated vaccines viz. budding pathogens in bulk (biosafety level 3) and complete sterilization of the grown pathogens. Successful sterilization of SARS-CoV, in bulk has been demonstrated with the help of UV radiations [118]. During the last two decades many investigators have demonstrated that inactivated whole SARS-CoV can induce SARS-CoV neutralizing antibody [65,117,119–122] but there have no study been reported against the live SARS-CoV challenge. A study in BALB/c mice, WKV vaccine was observed to provide resistance by ceasing the multiplication of pulmonary SARS-CoV, however immunological responses are yet to be verified [123]. Further, β-propiolactone inactivated WKV SARS-CoV (Tor-2 strain) reported to be a potential vaccine candidate as it induced neutralizing antibodies and was able to minimize the virus load in the pulmonary tract of the mouse model [87]. However, there are less evidences of the mouse models being infected by such a diseases clinically, thus studying models where this strain is more virulent and sustainable is imperative. So WKV has also been tested in ferrets, a model which show significant lung pathology and clinical sign [89,90]. Formalin inactivated Urbani strain of SARS-CoV did not demonstrated strong immune response [124] but β-propiolactone inactivated WKV SARS-CoV induced significant neutralizing antibody and reduced virus replication in respiratory tract of ferrets [89]. Rhesus monkeys were also been tested for the formaldehyde inactivated SARS-CoV vaccines and shown to be safe and immunogenic [125]. WKV SARS-CoV has also been tested in humans and found to be safe and elicited SARS-CoV specific neutralizing antibodies, however the efficiency the vaccine in humans yet to be reported [126]. These data suggested that WKV vaccines are safe and they are able to induce SARS-CoV specific neutralizing antibodies so inactivated viral vaccines could also be evaluated as potential vaccine candidate against SARS-CoV-2.

4. Conclusion

Many countries are affected by the rapid outbreak of SAR-CoV-2 infections so there is an urgent need to develop a safe and effective SARCoV-2 vaccine. Various research groups around the world have been able to start SAR-CoV-2 vaccine development by gaining knowledge from SARS and MERS vaccines development strategies. In this review we have extensively discussed the various vaccine targets or strategies based on SARS-CoV and MERS-CoV vaccine studies. The receptor-binding domain (RDB) of Spike protein (S) might be considered as a good vaccine antigen because it induces neutralizing antibodies that prevent host cell attachment and infection [127–129]. Further nucleic acid based vaccine, showed the most advance platform in response to emerging pathogens. DNA vaccine was the first vaccine candidate that entered in clinical trial (NCT02809443) [130] < 1-year after the Zika virus outbreak. Another nucleic acid-based vaccine, m-RNA vaccine can be considered as advanced vaccine technology because of its stability and protein translation efficiency so that it could induce robust immune responses [52,131]. Currently there are various biopharmaceutical companies or academic sectors are in the race to develop the prophylactic SAR-CoV-2 vaccine by using several platforms including mRNA, DNA, vaccinia or adenoviral vector and recombinant protein [112,132]. In order to make effective SAR-CoV-2 vaccine possible, target antigen(s)/nucleotide identification, immunization route, immune protection, suitable animal models, scalability, production facility, target product profile (TPP), outbreak forecasting and target population etc. are important parameters. As it is already known for some of the recent epidemics (Zika and Ebola) whose successful vaccine has not been developed yet. Lesson-learned from these epidemics in order to speed up the available vaccine during ongoing outbreak, preclinical studies of SAR-CoV-2 vaccine candidates may need to be performed in parallel with clinical trials. But in reality, SARS-CoV-2 vaccines will not be available for another 12–18 months because of the limitations such as unavailability of appropriate animal models to check the efficacy and toxicity before going for the clinical trials. Further it should comply with Good Manufacturing Practice (cGMP) to ensure the safety in humans. Post animal studies and cGMP clinical trial might be initiated in three phases (Phase I, II and III). Finally it require more time for the distribution and administration of the vaccine in a population currently naïve to SARS-CoV-2 infection. So there is a need of tight coordination and technology transfer among governments, regulatory agencies, pharmaceutical companies, and the World Health Organization (WHO), for the global production of effective vaccine against SARS-CoV-2 [112]. As the COVID-19 causes serious global health concerns, investigation into the characteristics of SARS-CoV-2, its interaction with the host immune responses could provide a clearer picture of how the pathogen causes diseases in some individuals and mild or no infection in others. Further, the evaluation of immunity and long-term memory of COVID-19 recovered individuals may be helpful in designing of a potential prophylactic and therapeutic agent not only against SARS-CoV-2 but other similar coronaviruses during future outbreaks.

Declaration of competing interest

The authors have declared no conflict of interest.

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