Rapid Response Research – SARS Coronavirus Vaccines and Application of Processes to Other Emerging Infectious Diseases

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Abstract: The near pandemic caused by the severe acute respiratory syndrome (SARS) emphasized that new and emerging infectious diseases not only continue to plague the world but also how the scientific community can unite to rapidly identify the causative agent and develop strategies such as vaccines to control its spread. The availability of the SARS coronavirus (SARS-CoV) genome sequence paved the way for the identification of recombinant vaccine candidates for SARS. Based on previous successful animal CoV vaccines, vaccinologists focused on the major CoV structural proteins such as the spike (S) and nucleocapsid (N) proteins as vaccine candidates. We will review the vaccine strategies SARS researchers have used and discuss current SARS animal models used for vaccine evaluation. The small number of SARS cases in 2004 has raised questions about whether SARS will return as a pandemic and the cost-effectiveness of testing a SARS vaccine in human clinical trials. Finally, the SARS outbreaks identified several gaps in the response to emerging infectious diseases. The SARS Accelerated Vaccine Initiative (SAVI) was established to provide rapid solutions to a public health emergency and to develop a new research paradigm for vaccine development for newly emerging and re-emerging infectious diseases.

Keywords: SARS, vaccines, coronavirus, neutralizing antibodies, immunology.

INTRODUCTION

Emerging infectious diseases present one of the most significant challenges facing the global public health community today. Infectious diseases are becoming increasingly prominent each year and are second only to cardiovascular diseases in the leading cause of death worldwide [1]. Many viral pathogens have emerged in recent decades including West Nile virus, HIV, new subtypes of influenza A, monkeypox and in late 2002, a novel coronavirus that is responsible for Severe Acute Respiratory Syndrome (SARS). An important hallmark of pandemics and of many less pathogenic emerging infectious diseases is that they are zoonotic in origin and are transmitted from animals to man either directly or *via* vectors [2]. Animals therefore represent an important reservoir for infectious diseases as in the case of SARS and influenza strains. RNA viruses are among the most prominent emerging pathogens because of their high mutation rates and subsequent ability to adapt quickly to changing environmental conditions or new hosts [3]. Several factors have led to the emergence of these infections in humans, including population growth, demographic changes, the industrialization of food production, globalization, international travel, microbial adaptation and decreased funding for public health measures in many regions [4]. Addressing these factors might help to reduce the rate of emergence of infectious diseases (currently estimated at one infectious disease per year).

The development of vaccines and implementation of vaccination programs are considered the most important medical contribution to humanity. For example, smallpox, polio, measles, and mumps have become non-existent or extremely rare in populations where vaccinations are widely available and accepted. To date, vaccination has reduced morbidity and mortality from infectious diseases more than any other specific medical intervention. As new and emerging infectious diseases appear, there will be a strong demand for new and innovative vaccines. Vaccine development is clearly a top priority in preventing disease outbreaks, either natural or man-made (bioterrorism). SARS coronavirus (SARS-CoV) is the first new pathogen to emerge in the 21st century but it will certainly not be the last.

The recent SARS pandemic threat revealed how a newly evolved pathogen can rapidly spread throughout the world and how the global community can unite to identify the causative agent and control its spread. In this review, we will describe the features of the SARS-CoV, the rationale for the approaches used in the development of vaccines for SARS, and how the vaccine lessons learned from SARS can be applied to new and emerging infectious diseases.

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BRIEF HISTORY OF SARS

The first cases of SARS were identified in November 2002 in Guangdong Province, China and by April 2003, the epidemic had spread worldwide. The outbreaks of SARS in 2002/2003 resulted in significant morbidity (8098 cases) and mortality (774 deaths) along with a global economic loss of greater than $90 billion worldwide [5]. Canada was particularly affected by SARS with 251 cases, 43 deaths and the issue of a World Travel Health Advisory [5, 6]. The causative agent of SARS has now been identified as the SARS-CoV both by genome sequencing of isolates from SARS patients and by experimental infection of cynomolgus macaques to fulfill Koch's postulates [7-12]. It is thought that the SARS-CoV in humans likely resulted from zoonotic transmission from wild game markets in Guandong, China [13] or other unidentified animal reservoirs [14, 15]. SARS-CoV-like viruses have been isolated from Himalayan palm civets and other animals found in live-animal market in Guangdong [13]. Sequence analysis of the SARS-CoV genome indicates that it evolved as a separate lineage and was not simply derived from a recombination of other known coronaviruses [9, 11]. Phylogenetic analysis of coronavirus sequences suggested that it was not closely related to the three previously identified serotypes (groups 1, 2 and 3) and as a result, it was proposed that the SARS-CoV be defined as a fourth class of coronavirus [9, 11]. Furthermore, the lack of antibodies to SARS-CoV in human populations was a strong indication that this was a new human virus [8, 14]. Ecologic changes, along with increasing contact between human and animal disease reservoirs may have contributed to the emergence of SARS. Prior to SARS, there had been little public interest in coronaviruses as other human coronaviruses cause only minor human diseases [16]. A new group 1 coronavirus, NL-63, has since been identified to cause bronchiolitis and pneumonia in humans [17, 18].

SARS is primarily a respiratory disease with the highest concentration of the SARS-CoV found in the respiratory tract [7, 8, 10] although this virus is also detectable in other organs, tissues as well as in stool [19-21]. The incubation period for the disease ranges from 2 to 10 days and infectivity is maximal during the second week of disease [22, 23]. The disease is characterized by fever, chills, malaise, dyspnea, cough, diarrhea and pneumonia [7, 8, 10]. Diffuse alveolar damage along with inflammatory cell infiltrate consisting particularly of macrophages are hallmarks found in SARS patients [24]. The fever of most patients settle within two weeks which is accompanied by resolution of chest symptoms and radiologic changes [7, 8, 10, 25, 26]. The major mode of transmission of the SARS-CoV is believed to be through droplet spread [11, 27] although SARS-CoV can remain viable when dried on surfaces for up to six days [28]. The majority of SARS patients are adults with only a few cases in children aged 15 or younger [22, 23, 29]. The overall case fatality rate is about 10% [22, 30].

IMMUNOLOGIC RESPONSE TO SARS-CoV

Both humoral and cellular immune responses have been shown to be protective against animal coronaviruses. Although immune correlates of protection against SARS-CoV have not been completely defined, there is evidence to suggest that a SARS-CoV vaccine would work in humans. Patients convalescing from SARS usually develop high titres of neutralizing antibodies [31] and the appearance of these antibodies typically coincide with the onset of resolution of the SARS pneumonia [32, 33]. Serum antibodies appear typically around 20 days after the onset of disease with the appearance first of IgM antibodies followed quickly by IgG antibodies which persist for greater than 4 months [34]. Interestingly, in another study of SARS patients, IgM antibodies to SARS-CoV nucleocapsid protein were detectable later than IgG antibodies, a phenomenon in contrast to that observed in most other pathogens [35]. With other coronaviruses, there is an inverse relationship between disease severity and pre-existing serum antibodies [36]. These studies indicate that neutralizing antibodies are likely to be important in protection against SARS. There is evidence suggesting that cellular immune responses are also important for protection against SARS-CoV. For instance, low concentrations of $CD4^+$ and $CD8^+$ T cells during a SARS infection are correlated with increased disease severity and mortality [37]. Furthermore, there are reports of protective cell-mediated responses elicited by non-surface exposed viral antigens. These include the nucleocapsid (N) protein of porcine transmissible gastroenteritis virus (TGEV) [38] and avian infectious bronchitis coronaviruses [39]. In the latter instance, serum antibody levels did not correlate with protection (although chickens possess a much more limited antibody repertoire than mammals) and adoptive transfer of IBV-infection-induced $CD8^+$ T cells protected chicks from challenge infection [39]. The kinetics of virus clearance in mice was also delayed in experiments in which $CD4^+$ or $CD8^+$ T cells were depleted prior to infection with murine hepatitis virus (MHV) [40, 41]. Specific human leukocyte antigen (HLA) class I alleles have been correlated with SARS susceptibility, indicating the involvement of $CD8^+$ T cells [42]. Collectively, these data suggest that a vaccine for SARS-CoV would ideally induce both neutralizing antibody responses and cell-mediated immunity to confer protection against SARS-CoV.

It has been hypothesized that SARS is a disease of cytokine dysregulation [19, 43, 44]. The progression of SARS to respiratory failure does not appear to be explained by uncontrolled viral replication, but due to immunopathological damage as a result of an over overexuberant host response [19]. The similarity of both pathological and clinical changes in SARS-CoV and H5N1 pneumonia suggests that pro-inflammatory cytokines released by stimulated macrophages in the alveoli may have a prominent role in the pathogenesis of SARS as well as influenza pneumonia and results from cytokine dysregulation [24]. A number of pro-inflammatory cytokines and chemokines have been detected in the plasma of SARS patient. In particular, TNF-α, TGF-β, IL-16, IL-13 and CXCL10 are found at high levels during the initial phase of the illness while TNF-α and IL-16 are elevated during the recovery phase[1] [45]. However, a recent study has shown that

[1]Cameron MJ, Persad D, Danesh A and Kelvin DJ. Cytokine and chemokine inflammatory loops and SARS. World Vaccine Congress Meeting, Montreal, 2004

the only cytokine or chemokine significantly increased in 100% of SARS cases within 48 h of onset was CXCL10, also known as IP10[1]. The authors concluded that elevated and sustained levels of CXCL10 and poor outcome in SARS patients may be the result of CXCL10-driven attraction of autoinflammatory cells or failure of the immune system to clear SARS-CoV through direct T cell killing[1]. The presence of rapidly replicating SARS-CoV in the lungs would stimulate monocytes, lymphocytes and epithelial cells, leading to the massive production of pro-inflammatory cytokines resulting in tissue injury and chemotaxis of white cells to the site of infection [5]. These white blood cells, particularly natural killer cells, would then serve to inhibit replication of the SARS-CoV. A recent study showed that the SARS-CoV can infect and activate human peripheral blood mononuclear cells with a consequent increase in chemokines (e.g. IL-8) involved in trafficking and mobilization of particularly monocyte/macrophage lineage cells [46]. The authors hypothesized that the pulmonary damage in SARS may not be a direct effect of the SARS-CoV on the alveoli, but represents the effects of cytokines induced by activated monocytes/macrophages that are recruited to the site [46]. This is consistent with the observation that inflammatory cells such as macrophages accumulate in the lung during the peak of the disease [44]. The role of cytokine dysregulation in SARS is further supported by the frequent use of high dose corticosteroids in the treatment of SARS [14, 19].

POTENTIAL FOR VACCINE DEVELOPMENT

The sequencing of the SARS-CoV genome was a key to the development of many diagnostic tests for SARS infections in humans and other animal hosts. More importantly, the sequence information was instrumental in the identification of potential viral antigens for recombinant vaccine development. In 2003, the World Health Organization (WHO) called for the development of SARS vaccines.

There are several lines of evidence that a vaccine for SARS-CoV could be developed with a high likelihood for success. Vaccines have been developed for several animal coronaviruses including chickens, bovine, canine, feline and porcine coronaviruses, with vaccines against infectious bronchitis of chickens being arguably the most successful [39]. Data from these vaccines provide much of our current knowledge about the efficacy of anti-coronavirus vaccines. Live attenuated coronaviruses, killed coronaviruses, DNA vaccines and recombinant viral vector vaccines have all been used to successfully vaccinate animals [39, 47, 48]. Furthermore, data show that a large number of patients infected with the SARS-CoV do recover from the infection. Immunogloblin G seroconversion starts at around day 10 and appears to correlate with a drop in viral load, which occurs between days 10 and 15 [14, 19]. Neutralizing antibodies have also been detected in the convalescent sera of SARS patients [49, 50]. This suggests that the body's immune system is capable of containing the virus and that a vaccine for SARS prevention is possible. Understanding the immune responses of convalescent patients may provide important clues as to the correlates of immunity needed to direct vaccine development [51]. The most notable concern regarding animal coronavirus vaccines has been the enhancement of viral disease by antibodies to epitopes of the spike glycoprotein which complexes with the virus to enhance their infectivity. This has been observed only in feline coronaviruses where there is disease exacerbation in vaccinated cats upon challenge with live virus [52-54] but it is important to ensure that a vaccine against SARS-CoV does not enhance disease.

The other key to the development of a successful SARS vaccine is the availability of relevant animal models to evaluate SARS vaccine candidates. Several animal models have been described for SARS including macaques [25, 55, 56], mice [57], ferrets [58], and more recently hamsters [59,60]. Cynomolgus macaques were the first animal models to be described for SARS. Some cynomolgus macaques infected intratracheally with SARS-CoV developed a rash, respiratory distress and lung pathology similar to biopsied lung tissue from SARS patients, thus fulfilling Koch's postulates and providing a potential animal model for vaccine evaluation [25, 55]. However, at least three North American laboratories have had little success in observing lung pathology and severe clinical signs in macaques after live SARS-CoV challenge [61-63]. Factors such as the dose or strain of SARS-CoV, the species of macaques used, the route of SARS-CoV administration and the day of autopsy may have accounted for the variability among the laboratories [64]. The WHO held a meeting in Rotterdam in February 2004 to discuss standardization of conditions for SARS-CoV challenge in different laboratories before non-human primates could be used for vaccine testing [64]. Other animal models such as ferrets support viral replication and demonstrate some level of lung pathology similar to humans and therefore, represent an alternative inexpensive model for vaccine testing [58]. Finally, some strains of mice, such as BALB/c and C57BL/6, have been shown to support some SARS-CoV replication but do not demonstrate the significant pathology or clinical disease observed in humans [57, 65]. Recently, 129SvEv or Stat1$^{-/-}$ mice given an intranasal dose of SARS-CoV were reported to develop self-limited bronchiolitis [66]. In the same study, only Stat1 $^{-/-}$ mice were found to progress to interstitial pneumonia and mediastinitis, although there was no evidence of the diffuse alveolar damage often seen in humans with SARS [66]. Therefore, despite the spectrum of animal models, no single animal species has been shown to reproduce all of the clinical signs and lethality observed in humans infected by the SARS-CoV.

POTENTIAL SARS-CoV TARGETS AS VACCINE CANDIDATES

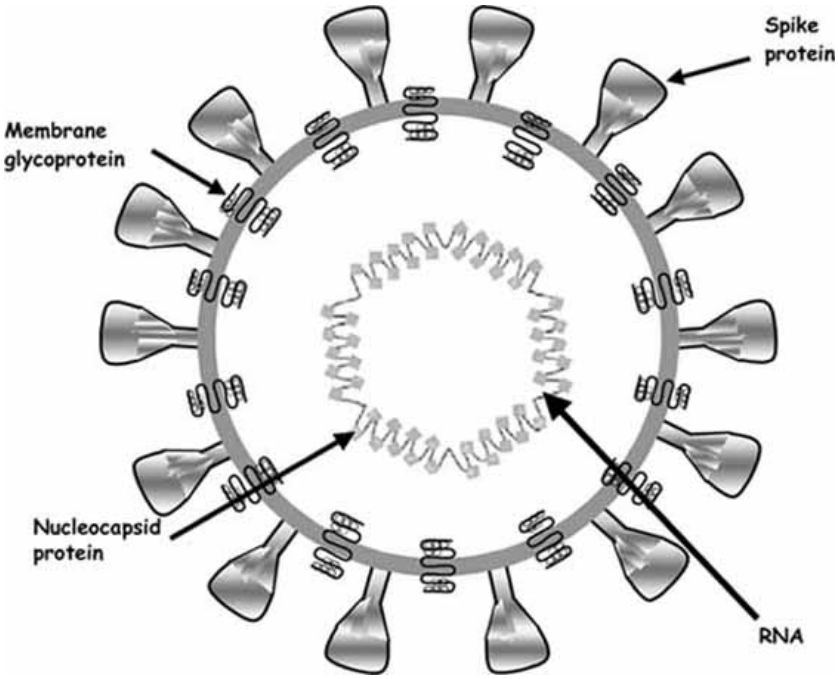
The sequencing of the SARS-CoV genome was the first step in identifying potential recombinant vaccine candidates for SARS. The Michael Smith Genome Sciences Centre in Vancouver, in collaboration with the British Columbia Centre for Disease Control, was able to dedicate their entire facility to the rapid sequencing of the SARS clinical strain

[1]Cameron MJ, Persad D, Danesh A and Kelvin DJ. Cytokine and chemokine inflammatory loops and SARS. World Vaccine Congress Meeting, Montreal, 2004

Toronto-2 (Tor-2) within 6 days of receiving viral nucleic acid [9]. Shortly thereafter, several other groups also reported genome sequences for SARS-CoV [11, 67]. The availability of the SARS-CoV genome sequence along with success with previously developed animal coronavirus vaccines suggested many potential strategies that could be considered for a SARS-CoV vaccine. As a result, full-length cDNA clones of SARS-CoV proteins have been of value to generate viral vectored vaccines or recombinant proteins.

Like its relatives, SARS-CoV is a plus-sense single-stranded RNA virus with a 30-kb genome with a 5´-cap structure and a 3´-polyadenylated tail [9, 11, 14]. The SARS-CoV has 11 major open reading frames (ORFs) encoding known coronavirus proteins including the replicase polyproteins, spike (S), small envelope (E), membrane (M) glycoproteins, nucleocapsid protein (N) and other unique proteins of unknown function [9, 11]. The non-structural proteins are encoded in the first two-thirds of the genome while the structural proteins such as spike, N, E and M are encoded in the 5-prime third of the genome [9, 11]. A schematic representation of the SARS-CoV along with some of the major structural proteins is shown in Fig. **1A**. Figure **1B** (depicted also in http://www.sarsresearch.ca) shows the topologic orientation of the SARS structural proteins in addition to the transmembrane domains that are potentially incorporated into the virion envelope. The spike protein is predicted to have the carboxy-terminal portion (amino acid residues 1218 to 1255) buried inside the virion, presumably inaccessible to antibodies. However, the amino-terminal domain of spike protein from amino acids 14 to 1196 (amino acids 1 to 13 is a cleaved signal peptide) is predicted to reside on the virion surface and therefore, may contain epitopes for protective antibodies. SARS-CoV E proteins are also exposed on the surface of virus-infected cells and virions while the location of the N protein is thought to be inside the viral envelope (Fig. **1A**) [68]. The spike protein has been shown to be important for binding to the angiotensin-converting enzyme- 2 (ACE2) on cellular membranes [69] whereas the E protein has been implicated in coronavirus assembly, M protein for virus assembly and budding, and N protein for viral RNA packaging [14, 70]. Coronavirus spike proteins have long been known to be a major determinant in coronavirus pathogenesis given that the viral protein interacts with cellular receptors as well as contains determinants important for eliciting a protective immune response [68, 70]. Therefore, this glycoprotein is important for viral entry into cells, tissue tropism, host range, and virulence [39, 71, 72]. Consequently, the spike protein is considered a prime target for coronavirus vaccine development. As a vaccine, the spike protein has been shown to provide protection against coronaviruses of different animal species [39, 68, 73, 74]. The SARS-CoV spike glycoprotein, like the spike of other animal coronaviruses, is comprised of an amino-terminal S1 subunit and carboxy-terminal S2 subunit [9, 11]. The SARS-CoV S1 subunit binds to the host cell receptor, angiotensin-converting enzyme-2 (ACE2) [69, 75, 76] while the S2 subunit is thought to play a role in membrane fusion [77, 78]. The binding domain of the spike protein for the ACE2 receptor has been defined between amino acid residues 318 to 510 of the S1 subunit [75]. A human monoclonal antibody to the S1 subunit inhibits and neutralizes SARS-CoV infection and efficiently inhibits syncytia formation through blocking of receptor binding [79]. The importance of the S1 subunit is further exemplified by the induction of protective immunity in chickens vaccinated with infectious bronchitis virus spike protein [39, 74, 80, 81]. Neutralizing antibody determinants have been found in both the S1 and S2 subunits of the spike protein, suggesting that these regions are good targets for the development of an effective SARS-CoV vaccine [75, 76, 82]. The spike protein is predicted to have 1182 exposed amino acids, which suggest that there may be many more B-cell epitopes than either E or M proteins, which have a predicted 42, and 26 exposed amino acids, respectively (Fig. **1B**). Besides spike protein, N protein has also been explored as another SARS-CoV target for vaccine development. Humoral immune responses against SARS-CoV N protein may not be useful given that the N-specific antibodies are not expected to penetrate the envelope to bind to the N protein [83]. A recent report also showed that antiserum to the SARS-CoV N protein does not contain neutralizing antibodies [84]. Although antibodies to coronavirus N proteins may not have virus neutralizing activity *in vitro*, there is evidence that the protein may provide *in vivo* protection by induction of cell-mediated immunity [68, 85, 86]. N protein has been shown to generate coronavirus-specific $CD8^+$ T cells [87-90] and provide protective effects in animals in response to infection by animal coronaviruses, including MHV and IBV [89,91,92]. Peptides to the SARS-CoV N protein have been found to induce a T-cell response in rhesus macaques [93]. Recently, it has been reported that the SARS-CoV N protein (expressed as a calreticulum-linked DNA vaccine) stimulates $CD8^+$ T cells resulting in a significant reduction in titer of challenging recombinant N vaccinia virus [83]. All of the above studies suggest that the SARS-CoV N protein may be important for the development of vaccines that stimulate cell-mediated immunity. Studies on SARS-CoV E and M proteins show that they do not induce any detectable serum SARS-CoV-neutralizing responses in hamsters [59] although a neutralizing antibody response for M protein has been demonstrated in rabbits [84]. Bioinformatics predicts that only the amino acids from 1 to 18 and 69 to 77 of M protein might be surface-exposed and thus offers a small target region for neutralizing antibodies (Fig. **1B**). The bulk of the M protein (120 amino acids) is hidden inside the virus particle, although this region may contain important T cell epitopes for cell-mediated immunity. Besides the above-mentioned SARS-CoV proteins, Fig. **1B** also shows that there may be other proteins incorporated in the virus membrane that could potentially serve as important protective vaccine targets for generating virus opsonizing or neutralizing antibodies. Studies with convalescent sera of SARS patients indicate that indeed, all of these proteins, with the exception of ORF 7 and 8 are recognized by patient antibody [94]. Whether these other predicted proteins are incorporated into the virus particle remains to be shown biochemically, especially for ORFs 4, 7 and 14, which show a weak prediction for a transmembrane spanning region. Collectively, the data indicate that among the structural proteins, the spike protein is the most important SARS-CoV protective antigen given its role in virus binding and fusion [59]. Other SARS-CoV targets, such as N and M proteins, may be important targets for vaccines that are based on cell-mediated immunity.

A



B

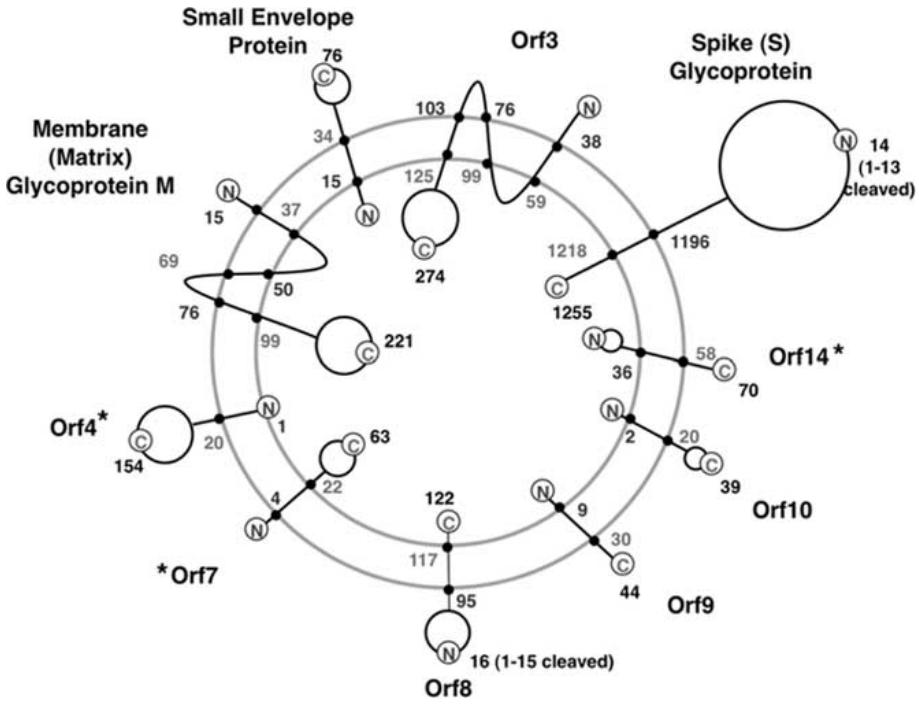


Fig. (1). A. Schematic representation of the severe acute respiratory syndrome coronavirus (SARS-CoV) particle. The positions of three of SARS-CoV structural proteins (spike, nucleocapsid and membrane) on SARS-CoV that were targeted for vaccine development are indicated. B. Diagram illustrating potential SARS-CoV membrane proteins. Structural proteins that have a predicted transmembrane domain are shown with the carboxy (C) and amino (N) orientation relative to the plasma membrane or viral envelope (whether these SARS proteins localize to the viral envelope is unknown). The locations of the large hydrophilic domains of the proteins are depicted as large circles. Numbers show the approximate locations of predicted transmembrane spanning regions and termini. The topologies and membrane predictions are discussed in more detail in [9].

* These proteins are weakly predicted to have transmembrane domains.

Table I. Vaccine Technologies Available for SARS Vaccine

Type of Vaccine	Advantages	Disadvantages
Inactivated viruses	· Full complement of virion proteins · Effective in inducing antibody responses	· Loss of structural integrity by inactivation method · Risk of infectious particles · Induces poor CTL response · Challenge for manufacturing under BSL-3, cGMP conditions
DNA vaccines	· Induces sustained humoral and cellular-mediated responses · Safe, low risk · Stable, easy to manufacture	· Protein expression in humans lower than observed in viral vectors · Potency in humans disappointing compared with animals
Viral vectored vaccines	· Induces strong CTL response and humoral immunity · Well-characterized genomes · Low cost · Ease of manufacturing and administration · Stability of freeze-dried vaccines · Can be used in prime-boost strategies · Different routes of administration (e.g. intranasal, intramuscular, etc.)	· Pre-existing immunity (adenovirus) · Limited cloning capacity (adenovirus) · Risk for immunocompromised individuals (adenovirus)
Recombinant proteins	· Induces potent humoral immunity · Safe, effective · Able to engineer proteins to increase stability and solubility · Different expression systems available (e.g. mammalian, yeast, bacteria, insect cells)	· Poor CTL response · Post-translational modifications may be required · Correct protein folding may be important · Poor expression for some viral proteins unless codon-optimized gene system used

CTL, cytotoxic T lymphocytes; cGMP, current General Manufacturing Practice; BSL-3, Biosafety Level 3.

SARS-CoV VACCINE STRATEGIES

Prior experience with other infectious diseases showed that vaccination would be one of the most effective interventions for controlling future SARS outbreaks. As a result, several laboratories have utilized different strategies for the development of a SARS vaccine. The pros and cons of each strategy are summarized in Table **I** and each of these strategies will be briefly reviewed here. For the reasons outlined above, most researchers have focused on a SARS vaccine based on the SARS-CoV spike or N protein as targets.

Whole-Killed Virus

As mentioned earlier, most of the current knowledge on coronavirus vaccines has been generated through vaccine studies of veterinary importance. Safe and effective whole-killed coronavirus vaccines have been successfully developed for canines [95] and bovines [96] and chickens although the success in chickens has been less than that observed with live attenuated vaccines [39]. The approach most commonly used in the poultry industry is first to prime chickens with live vaccine followed by a boost of inactivated vaccine [39]. The success of these animal coronavirus vaccines has led to the development of inactivated SARS-CoV vaccines by several laboratories and vaccine companies (summarized in Table **II**). Whole-killed virus vaccines are common in the animal food industry as they are safe and economical to produce [51]. Development of a whole-killed SARS-CoV vaccine is quick compared to other vaccine strategies and eliminates the need to determine which antigens might confer protection. One disadvantage to the whole-killed virus vaccine strategy is that the inactivation procedure may destroy the antigenicity of the viral proteins and that stringent quality control checks are frequently needed to ensure the elimination of any infectious particles during the preparation. Another drawback to whole-killed virus vaccines is that exact specifications of the vaccine composition and mechanisms of protective immunity as required by the Food and Drug Administration (FDA) and the World Health Organization (WHO), are difficult to meet [97]. Lastly, large-scale production of a Biosafety Level-3 pathogen is expensive and problematic by any standards.

The SARS Accelerated Vaccine Initiative (SAVI) has developed and tested the whole-killed virus vaccine (β-propiolactone-treated) as one of its strategies for generating a SARS-CoV vaccine (Table **II** and [64]). Administration of the whole-killed SARS-CoV vaccine and a booster dose into either mice or ferrets resulted in the induction of high titers of neutralizing antibodies (unpublished data). Similar levels of neutralizing antibodies induced by inactivated SARS-CoV have been found in BALB/c mice by others [98, 99]. More recently, Takasuka *et al*. [100] showed that subcutaneously injected UV-inactivated SARS-CoV vaccines elicited high levels of serum IgG but not IgA antibodies directed against spike and the nucleocapsid protein. In the same study, the inactivated virion was found to induce production of cytokines IL-2, IL-4, IL-5, IFN-γ and TNF-α from lymph node T cells, suggesting activation of T cells [100]. Despite several reports now describing the development of a whole-killed SARS-CoV vaccine, none have described whether these vaccines can protect against a live SARS-CoV challenge in a relevant SARS animal model such as ferrets or mice. At SAVI, we have recently completed studies showing that our inactivated SARS-CoV vaccine protects animals against live SARS-CoV challenge (manuscript in preparation).

DNA Vaccines

A main goal for the successful vaccination against infectious diseases like SARS is the stimulation of both

Table II. Candidate SARS-CoV Vaccines from Industry and Academic Laboratories

Developer	Vaccine Strategy	SARS-CoV Protein	Type of Immunity		Protective Immunity in Animals	References
			Neut. Ab	CMI		
McMaster Univ. (SAVI)	Adenovirus vector	S, N	+(I.M.)	+	+, SARS-CoV challenge in ferrets, mice	[64], www.savi-info.ca
Univ. of Pittsburgh	Adenovirus vector	S, N, M	+	+		[93]
NIAID	BHPIV3	S	+		+, SARS-CoV challenge in African green monkeys	[56]
Aventis Pasteur	Inactivated virus		NA	NA		[61]
Baxter Healthcare	Inactivated virus		NA	NA		[61]
Chiron Vaccines	Inactivated virus		NA	NA		[61]
National Institute of Infectious Diseases, Shinjuki-ku, Japan	Inactivated virus		+	+		[100]
Sinovac/CAMS	Inactivated virus		NA	NA		[61]
Univ. of British Columbia (SAVI)	Inactivated virus		+	+	+, SARS-CoV challenge in ferrets, mice	[64], www.savi-info.ca
Wuhan Institute of Virology, China	Inactivated virus		+		+, mice	[99]
Johns Hopkins University	Plasmid DNA	N	ND	+	+,Vaccinia virus N challenge	[83]
Nanjing Normal University, China	Plasmid DNA	N	ND	+	+, Vaccinia virus N challenge	[105]
U.S. Vaccine Research Center	Plasmid DNA	S	+	+	+, SARS-CoV challenge in BALB/c mice	[103]
National Microbiology Laboratory, Canada	Vaccinia virus vector	S	+	ND	Enhanced hepatitis in ferrets	[112]
NIAID	Vaccinia virus vector	S	+		+, SARS-CoV challenge in BALB/c mice	[111]
Robarts Research Institute, (SAVI)	Vaccinia virus vector	S,N	ND	ND		[64], www.savi-info.ca
Peking University, China	Recombinant protein	S	+		+, SARS pseudovirus challenge in rabbits, mice	[82]
Protein Sciences	Recombinant protein	S	+	NA		[61]
Univ. of Massachusetts	Recombinant protein	S	NA	NA		[76]
Univ. of Toronto (SAVI)	Recombinant protein	S	+	ND		[64], www.savi-info.ca
ID Biomedical Corp.	Proteosome	S				www.idbiomedical.com

Neut. Ab, neutralizing antibody; CMI, cell-mediated immunity; S, spike; N, nucleocapsid; M, membrane; I.M., intramuscular; ND, not determined; NA, not available; BHPIV3, bovine human parainfluenza virus.
SAVI, SARS Accelerated Vaccine Initiative; NIAID, National Institute of Allergy and Infectious Diseases.

humoral and cellular immune responses for protection. Many currently licensed vaccines are able to induce antibody responses but only vaccines composed of live recombinant or attenuated organisms induce cellular immunity efficiently [101]. DNA vaccines, comprised of plasmid DNA encoding proteins from pathogens, allergens, and tumors, which are then taken up by the nucleus of cells, have been evaluated as prophylactic and therapeutic treatments for infectious diseases, allergies and cancer. Over the last decade, plasmid DNA vaccines have been demonstrated to induce both humoral and cellular immune responses, the latter due to the mimicking of the effects of live-attenuated viruses in the ability to induce major histocompatibility complex (MHC) class I-restricted $CD8^+$ T-cell responses [101]. Furthermore, the stability, simplicity, safety, and ease of manufacture make DNA vaccines an alternative to the use of live vaccines [101]. DNA vaccines have been shown to induce sustained humoral and cellular immune responses even in the absence of detectable antigen [101, 102]. DNA vaccines have been made for a variety of infectious diseases including influenza, HIV and West Nile virus [101]. Therefore, it is no surprise that several groups have used this approach for the generation of SARS-CoV vaccine candidates. Several DNA vaccines have been reported for SARS-CoV proteins, including spike [103, 104] and nucleocapsid protein [83, 105]. Yang *et al*. [103] showed that a spike DNA vaccine induced both T cell and neutralizing antibody responses. SARS-CoV replication in the lungs of vaccinated mice was reduced by six orders of magnitude compared to control animals in their study [103]. Moreover, using T-cell depletion and immune IgG passive transfer, the authors showed that protective immunity was mediated by a humoral and not by a T-cell-dependent immune mechanism [103]. The latter result suggests that antibodies against the SARS-CoV spike glycoprotein alone can protect against SARS-CoV challenge and do not enhance infection in the animal model [103]. Kim *et al*. [83] showed that DNA vaccination with SARS-CoV N protein linked to calreticulin (CT) not only generated potent N-specific humoral and T-cell-mediated immune responses but also significantly reduced the titer of challenging vaccinia virus expressing the N protein.

Although DNA vaccines show great promise in pre-clinical models, their ability to protect against human diseases has yet to be established and clinical studies results have generally been disappointing. DNA vaccines do not achieve the levels of protein expression as those observed using viral vectors and therefore, may result in a sub-optimal immune response. One solution may be a heterologous

prime-boost combination with either inactivated viral vaccine candidates or viral vectors such as that recently described in humans using a malaria vaccine [106]. This combination results in a stronger CTL response than can be achieved by priming and boosting with the same agent [107]. However, pre-clinical trial studies coupled with large-scale human trials will be needed in order to validate the use of this new vaccine approach.

Virus Vectored SARS-CoV Vaccines

Among the new vehicles for antigen delivery, live recombinant viruses have several features that make them extremely efficient in inducing both B- and T-cell-mediated immune responses. Viral proteins can act as a strong immunization adjuvant, and recombinant vaccines can be selected or engineered to infect antigen-presenting cells directly and efficiently [108]. Therefore, recombinant viruses can express the foreign protein target endogenously in the cytoplasm where it can be processed for presentation in the context of MHC Class I to $CD8^+$ T cells for efficient priming of a cytotoxic T cell response [108]. As a result, recombinant viruses do result in activation of cellular immunity necessary for elimination of infected cells. Some of the other advantages for the use of adenoviruses as vaccines include the ability to administer the vaccine orally and the well-established techniques already in place for making adenoviral recombinants due to the well-characterized genome of adenoviruses (reviewed in [109]). The major disadvantages for the use of adenoviruses as vectors compared to other viral systems are their limited cloning capacity, pre-existing adenovirus-specific immunity [108], and the restricted host range of human adenoviruses which makes vaccine testing in animals a challenge [110]. Several recombinant viral strategies have been designed for SARS-CoV vaccines including adenovirus, vaccinia virus and parainfluenza virus. Each strategy, which will be discussed in this section, has been used to express viral proteins with the goal of inducing both humoral and cell-mediated immunity.

As part of the mandate by SAVI to develop a SARS-CoV vaccine, recombinant adenovirus constructs containing genes for expression of either SARS-CoV spike or nucleocapsid (N) proteins were developed at McMaster University in Ontario (Table **II**). Results show that the administration of a combination of recombinant adenovirus spike and N proteins to either ferrets or mice were able to induce a neutralizing antibody response in animals (manuscript in preparation). Besides SAVI, another laboratory has also developed recombinant adenoviruses containing SARS genes. Gao *et al*. [93] reported that when they immunized rhesus macaques intramuscularly with a combination of three Ad5-SARS-CoV adenovirus-based vectors (N, spike, and M protein), the vaccinated animals all had antibody responses against the spike S1 fragment along with T-cell responses to the N protein. The authors, however, did not report whether the vaccine combination was able to protect the rhesus macaques against live SARS-CoV challenge in their study.

One caveat to using recombinant adenovirus for a SARS-CoV vaccine is that the immune responses may be generated against the vector itself. In testing efficacy of vaccines consisting of recombinant adenovirus expressing SARS-CoV proteins, we have observed that the adenovirus vector alone may induce general anti-viral immunity that obscures the effects of the specific immune response to SARS-CoV proteins (unpublished data). Furthermore, a large percentage of the human population has pre-existing immunity against the vector due to naturally occurring infections. As a result, the immunogenicity and clinical utility of recombinant vector-based vaccines (e.g. Ad5) for SARS-CoV or antigens for other infectious diseases may be limited by the high prevalence of pre-existing immunity to Ad5. One way to circumvent this problem of pre-existing immunity is the use of prime-boost protocols where a different vector (e.g. DNA vaccine) is used to prime the immune response followed by a boost with an antigen encoded by either a recombinant adenovirus or recombinant MVA virus [108]. Finally, vaccination with a recombinant adenovirus vector SARS vaccine may be problematic for immunocompromised individuals.

Besides adenoviruses, recombinant viruses for SARS-CoV proteins have also been constructed from the modified VV Ankara strain (MVA). Poxvirus recombinants are good candidates as vaccine vectors due to their low cost, the stability of freeze-dried vaccines, ease of manufacture and administration, cytoplasmic site of gene expression, ability to induce cellular immune responses with long lasting immunity and the capacity for cloning large foreign genes [108]. MVA-based vaccines also do not replicate efficiently in mammalian cells and can be used in immunocompromised patients [108]. Recently, the SARS-CoV spike protein expressed in MVA was found to induce neutralizing antibodies to SARS-CoV and elicit protective immunity in mice, as demonstrated by reduced viral replication in the upper and lower respiratory tract after challenge [111]. Both intranasal and intramuscular routes of administration of the MVA SARS-CoV spike vaccine (MVA/S) were effective in reducing SARS-CoV titers in the lungs and nasal turbinates of mice [111]. No enhanced virus replication or enhanced disease was found in the mice immunized with MVA/S before challenge with SARS-CoV [111]. However, it has recently been reported that administration of MVA/S but not MVA vector alone into ferrets followed by live SARS-CoV challenge resulted in enhanced hepatitis [112]. The authors hypothesized that the liver pathology observed in ferrets was likely due to immune responses from the vaccination of the spike protein [61, 112]. The lack of liver disease in the mouse study by Bisht *et al*. [111] following MVA/S vaccination suggests that there may be interspecies differences in immune responses to MVA/S in mice and ferrets. Therefore, it is recommended that signs of liver disease after spike protein vaccination be monitored closely following the SARS-CoV post-challenge period in all animal models.

Other investigators have focused on intranasal live-attenuated viral vectors as a means of inducing sustainable protective immunity *via* mucosal route [113]. Bukreyev *et al*. [56] showed in African green monkeys that a single intranasal administration of spike protein expressed in attenuated bovine/human parainfluenza virus type 3 (BHPIV3) induced neutralizing antibodies to SARS-CoV and significantly reduced viral titers in the respiratory tract

after live viral challenge. Therefore, this SARS-CoV vaccine strategy is seen to have several advantages: 1). counteract against the virus at the level of mucosal membranes of the eyes and respiratory tract, thought to be the primary mode of transmission for the virus [114, 115] and 2). mucosal immunization with the BHPIV3/S is expected to induce both systemic as well as local immunity [56] and 3). The vaccine would be considered safe and effective for infants and young children [56]. Since coronaviruses commonly infect respiratory or enteric mucosal areas, the induction of mucosal immunity may be a key strategy in eliciting protection against these viruses [68].

Recombinant Protein Vaccines

Molecular biology and genetic engineering have provided vaccine development with useful technologies for recombinant protein production. Bacterial polysaccharides and viral surface proteins as well as detoxified toxins are some examples of subunit immunogens used as vaccines. The main advantage of recombinant subunit vaccines is that the pathogen is entirely excluded from the vaccine production process, thus eliminating safety risks at the stages of manufacturing and vaccine administration. Furthermore, the genes encoding the antigens can be genetically manipulated to increase protein solubility and stability [97]. Major drawbacks of recombinant proteins include the lower immunogenicity compared with viral vectored vaccines and the inability to generate a good CTL response through endogenous antigen synthesis and presentation in MHC Class I [108]. In addition, viral proteins such as the SARS-CoV spike protein are poorly expressed in mammalian cells due to incompatible codon usage unless a synthetic codon-optimized gene system is used [76].

A variety of approaches including bacteria [82, 116, 117], mammalian cells [61, 75, 76, 118] and insect cells (unpublished data) have been used to express the recombinant SARS-CoV spike protein. The protein expressed from mammalian and bacterial systems have been shown to induce neutralizing antibodies to SARS-CoV [82, 117] as well as bind to the ACE2 functional receptor [75, 76, 116, 118]. Using purified S1 or S2 fragments, the minimal ACE2 receptor binding site on the spike protein resides within amino acid residues 318 to 510 [75, 76]. Neutralizing antibody determinants have been located on both the S1 [76, 117] and S2 [82] fragments of SARS-CoV of spike protein, suggesting that these regions are important epitopes as vaccine candidates. A current strategy for the generation of soluble SARS-CoV spike protein fragments is the removal of the gene segment encoding the carboxy-terminal hydrophobic transmembrane domain [76, 103]. This results in secretion of the spike fragment into the growth medium, with subsequent spike purification by affinity chromatography [76]. At SAVI, we have expressed full-length SARS-CoV spike protein in insect cells but solubility and protein aggregation appears to be a complicating problem (unpublished data).

To date, there are no published reports of a purified recombinant spike protein, either full-length or truncated protein, being evaluated in a live SARS-CoV challenge model. Recombinant SARS-CoV spike protein given as a SARS vaccine would be expected to protect vaccinated animals by induction of neutralizing antibodies [69,76]. Further validation that spike protein may be a good vaccine candidate against SARS-CoV infection is that a human monoclonal antibody (80R) against the S1 domain of spike is able to neutralize SARS-CoV infection of cells and block syncytia formation [79]. The main concern in using spike recombinant protein as a SARS-CoV vaccine relates to antibody-dependent immune enhancement of the disease (discussed above) and the possible emergence of new SARS-CoV strains with a spike protein different from those strains involved in the 2002/2003 outbreak. The spike gene was already found to have mutated specifically and rapidly after SARS entered the human population, apparently in response to selective pressure [119]. Research has shown that with infectious bronchitis virus, a 5% change in S1 amino acid can significantly reduce cross-protection of coronavirus vaccines [39]. Thus, the recombinant protein may have drawbacks as a vaccine candidate should there be mutations and recombinations to form new SARS-CoV strains. As a result, amino acid differences between spike proteins of SARS-CoV strains from different geographical regions should be examined prior to committing spike protein as a SARS-CoV vaccine candidate.

SARS ACCELERATED VACCINE INITIATIVE (SAVI); A NEW PARADIGM FOR VACCINE DEVELOPMENT

The near pandemic nature of SARS led the Provincial British Columbia Government to provide Cdn $2.6 million in April 2003 to establish the SARS Accelerated Vaccine Initiative (SAVI) whose mandate was two-fold: 1. to develop a human SARS-CoV vaccine as quickly as possible and 2. to develop a new model for scientific collaboration to address serious emerging infectious diseases [64]. SAVI (website: http://www.savi-info.ca) was established to apply rapid response research to a public health issue (reviewed in [64]). The SARS outbreak in 2002/2003 required development of rapid research response mechanisms to accelerate the development of a SARS vaccine and to apply these concepts to other new and emerging infectious diseases. A senior management committee was established that had significant expertise in the areas of animal coronavirus vaccines, immunology, epidemiology, grant-funding mechanisms, clinical trials and regulatory affairs. An emergency management system was quickly adopted to solve scientific problems in parallel instead of in sequence. Shortly after the Michael Smith Genome Sciences Centre in Vancouver sequenced the SARS-CoV genome, research strategies were executed in parallel including the identification of SARS-CoV vaccine candidates, development of immunological and virologic assays, propagation of SARS-CoV in tissue culture, development of clinical trial protocols and consultation with regulatory officials such as Health Canada- all with the single goal of developing a SARS vaccine as quickly as possible (see Fig. **2**). A SARS-CoV website (http://www.sarsresearch.ca) was designed with news, a discussion forum, reviews, analysis of the SARS-CoV genome and proteins, and advanced software and databases developed for coronaviruses. Funding mechanisms for these parallel strategies were quickly

allocated upon peer review of two-page budgeted grant proposals within a 2-day period. SAVI adopted 4 strategies in parallel for a SARS-CoV vaccine including a whole-killed virus vaccine, viral vectored vaccines consisting of recombinant adenovirus and vaccinia virus, and recombinant SARS-CoV proteins (Table **II**). Each of the above-mentioned strategies was developed by laboratories with specific expertise in that approach. The development of four SARS-CoV vaccines in parallel afforded the opportunity to go into a prime-boost strategy if necessary. At the same time, SAVI consulted with regulatory officials at Health Canada early on in the vaccine development process to define the regulatory guidelines needed for rapid approval of vaccines for current Good Manufacturing Practices (cGMP) production and human clinical trial studies (Fig. **2**). These included knowledge of clinically approved cell lines for vaccine production, identification of vectors and plasmids suitable for human vaccines and an understanding of the vaccine manufacturing process using current cGMP. Using this new paradigm for rapid research response, SAVI was able to develop 4 SARS-CoV vaccines within 6 months and evaluate two in head-to-head studies in two animal models for SARS in a total time of 15 months. Typically, the basic research for antigen identification and vaccine development and testing in animal trials process would take 5 to 10 years. From this new scientific collaboration many lessons were learned for developing rapid response to new and emerging infectious diseases. International cooperation and coordination are needed to avoid duplication of efforts and to maximize use of resources for tasks such as disease tracking and sharing of scientific knowledge. Furthermore, emergency management plans must be in place to quickly identify (e.g. genome sequencing), track, and develop therapeutic and diagnostic strategies for newly emerging pathogens. More downstream processes of vaccine development must also be heavily considered including manufacturing and commercialization of SARS vaccines. One clear shortcoming has been identified; there is a shortage of Biosafety Level-3 (BSL-3) facilities in North America both for animal testing and for propagating live SARS-CoV needed for cGMP production of a whole-killed virus vaccine. However, the rapid success of various SARS research programs has shown that rapid research response can be applied quickly to identify a new pathogen, sequence its genome, and develop preventative, therapeutic and diagnostic approaches in an accelerated and cooperative manner. The response to SARS has shown that vaccines can be rapidly developed in a cooperative and collaborative manner for any newly emerging or re-emerging infectious disease.

FUTURE OF SARS VACCINE AND SARS

As indicated in Table **II**, there are currently a number of different strategies being used by various academic laboratories and vaccine companies for the development of a SARS vaccine. Based on experience with animal coronavirus vaccines, a SARS-CoV vaccine that elicits both humoral and cell-mediated responses may be important to protect against SARS-CoV infection. For example, in mice challenged with the mouse hepatitis virus (MHV), a humoral response is important for reducing viral load but the actual eradication of the infection requires T cell help [51, 120]. Adoptive transfer of $CD8^+$ T cells resulted in protection and enhanced virus clearance in mice infected with mouse hepatitis virus infection [87, 121]. The inactivated SARS-CoV vaccine is the most straightforward approach. Earlier in 2004, Sinovac Biotech in China had initiated human phase I clinical trials with 36 healthy volunteers to evaluate the safety of this vaccine approach. The main concern associated with this approach is the ability to manufacture large quantities of SARS-CoV under cGMP and BSL-3 facilities, of which there are none in Canada and only a few in the United States or in the world. Performing rapid response research on highly infectious diseases would place a heavy burden on these facilities, particularly at a time where bioterrorism agents and newly emerging pathogens such as West Nile virus, avian flu virus and SARS-CoV together with the likely re-emergence of pandemic flu will result in renewed interest in vaccine development and manufacturing of these products. Both Aventis Pasteur and Baxter Healthcare are in the process of developing their own SARS-CoV inactivated vaccines after being awarded large contracts from the U.S. National Institute of Allergy and Infectious Diseases (NIAID). Besides the whole-killed virus vaccine strategy, Table **II** shows that others have focused primarily on the spike protein and, to a lesser extent, N protein as a target for SARS vaccine development [83, 103, 111]. The Vaccine Research Centre in Maryland has produced a DNA vaccine to SARS-CoV spike protein and is now working with Vical Inc. to produce enough doses for human clinical trials. Although this and other approaches (e.g. MVA spike) are technically feasible, the timelines for the development of any of these vaccines are lengthy [15]. There are also concerns from WHO officials that coronavirus mutations may make it difficult to focus on a single SARS-CoV vaccine based on spike protein [51]. It has been suggested that such an approach may require global surveillance of SARS-CoV to continually update the vaccine, much like the influenza virus [51]. In addition, the spike protein has been shown to elicit humoral immunity [75, 76, 82, 111, 122] and, as pointed out earlier, a combined humoral and cell-mediated immune response may be required for complete and efficient SARS-CoV clearance. Therefore, a prime-boost strategy consisting of viral vectors, DNA vaccines or recombinant protein might offer the advantages of generating both types of immune responses. Another strategy is the development of SARS-CoV vaccines that stimulate mucosal immunity to neutralize the virus at the site of infection [56].

A number of points need to be addressed before furthering the SARS-CoV vaccines into human clinical trials. The first relates to the relevance of vaccine evaluation in animal models developed thus far for SARS. As mentioned earlier, although the SARS-CoV can infect and replicate in many different animal species, none has developed the fulminant disease, clinical signs and death observed in human SARS. Ferrets, hamsters and non-human primates display enough viral replication and lung pathology that testing SARS-CoV vaccines in these animals should provide us with significant insights on vaccine efficacy [25, 58, 60]. The second point is that more studies are needed to address the antibody-dependent immune enhancement of disease as observed in vaccinated cats infected with feline infectious peritonitis virus [52-54]. The role of SARS-CoV

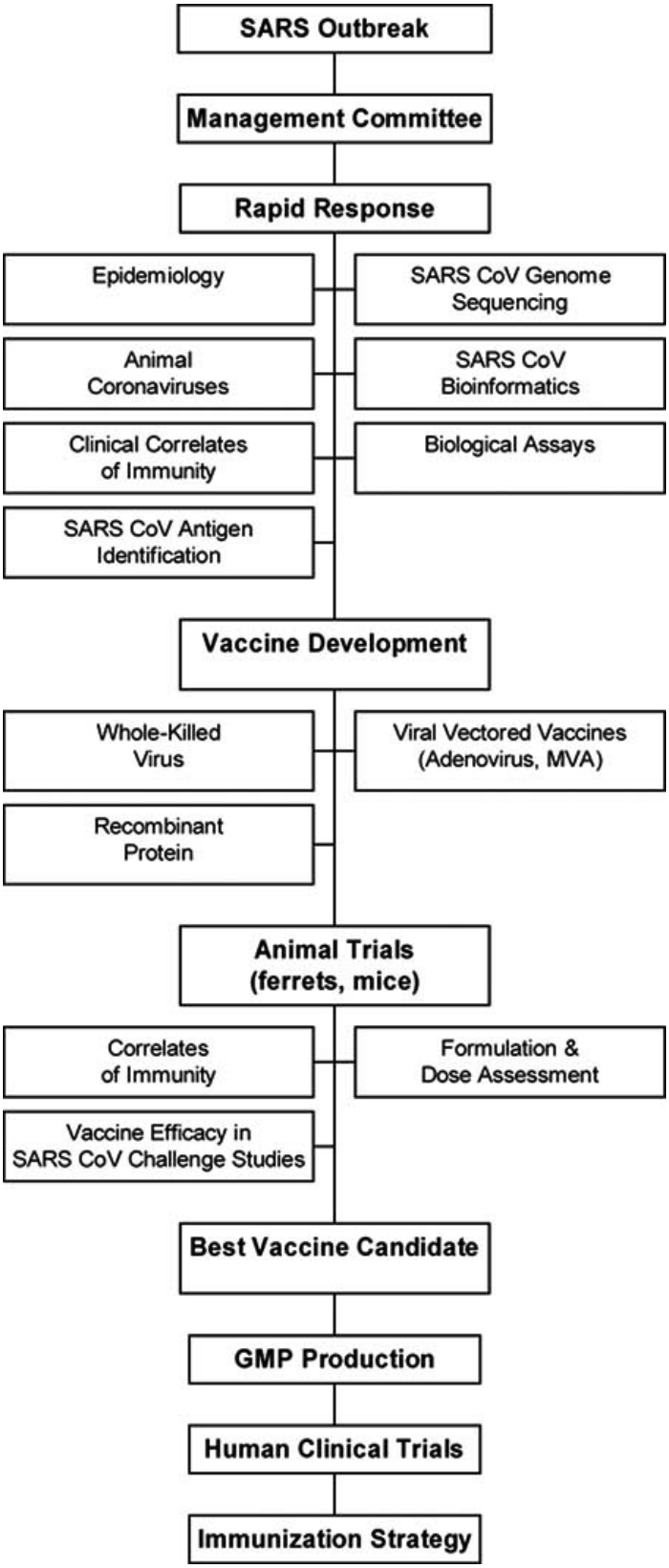


Fig. (2). Generic model describing the rapid research response utilized by SAVI for development of multiple vaccines for SARS. The outbreak of SARS led to the rapid development of an emergency management committee to coordinate research approaches in parallel. Using this information, various strategies were used for the development of vaccines based on SARS-CoV antigens. Multiple vaccines were compared in head-to-head studies in SARS animal models, with the best vaccine designated for cGMP production and human clinical trials.

antibodies need to be defined in their ability to exacerbate the disease before moving a vaccine to human clinical trials. The recent data from the National Microbiology Laboratory in Winnipeg, Canada using a MVA-based spike vaccine suggest that further studies may be needed to address liver pathology when ferrets are vaccinated and challenged with live SARS-CoV [61, 112]. These toxicology studies should be done in parallel to vaccine efficacy testing in animals as suggested by the WHO [61]. It has also been reported that cell-mediated immunity may also play a role in exacerbation of diseases in other coronaviruses. For example, a CD8+ T cell epitope on the surface of glycoprotein of murine hepatitis virus has been implicated in the demyelination of the brain and spinal cord of mice following infection [123, 124]. The last point relates to how SARS-CoV vaccines can be evaluated clinically given the lack of SARS cases globally this year. Phase I and II human clinical trials are designed to test the safety and immunogenicity of the vaccines in addition to identification of the correlates of immunity. Without an outbreak of SARS to test the efficacy of the vaccines in Phase III human trials, licensing of the vaccine may have to take place under emergency conditions under the FDA's "animal efficacy rule". The rule states that vaccines or other biological agents can be licensed for human use if the vaccine has demonstrated both human safety as well as efficacy in two species of animals upon infectious challenge (http://www.hhs.gov/nvpo/policy_reg.html). So far, no vaccines have been licensed under the animal efficacy rule.

A major issue that will impact directly on the speed of vaccine development is whether SARS will return and if so, when. There have been only a small number of cases of SARS in 2004, of which a few have been associated with negligence in laboratory handling of the virus. The WHO recently placed a warning that SARS could re-emerge in the winter of 2004 given that there is no evidence this respiratory disease has totally been eradicated in China. As long as animal reservoirs like civic cats, raccoon dogs and ferret badgers exist, there will always be the possibility of SARS returning in the coming months or years, perhaps in a seasonal pattern. Furthermore, retrospective serologic studies indicate that the ancestor to the SARS-CoV has been entering the human population from animals for several years so it seems likely this will continue [125]. Additionally, there was initially some concern that humans might develop chronic persistent SARS infections and may serve as a source of continuing SARS outbreaks [15]. Though most human coronaviruses cause short, self-limiting illnesses, most animal coronaviruses such as feline infectious peritonitis virus (FIPV) can cause persistent, asymptomatic infections and can shed virus as long as 7 months after infection [15, 126]. Fortunately, this concern has not been borne out with human SARS.

Who would be vaccinated should a SARS-CoV vaccine be developed and commercialized? It has been suggested that a ring vaccination strategy would be used for people who are at highest risk including healthcare employees, airline workers and individuals or communities exposed to SARS patients. Given that vaccine development can cost up to U.S. $500 million [127], many vaccine companies are not willing to invest heavily into the development and commercialization of a SARS vaccine given the low return-of-investment. Alternatives such as public health-industry partnerships will need to be formed where the public health sector laboratories and academic research facilities are involved in the initial R&D for vaccines while industry and government cooperate to sponsor cGMP vaccine production, human clinical trials and acceleration of the product into the market. Therefore, partnerships between corporate entities and research agencies are crucial in translating novel scientific findings from the laboratory to the clinic.

A typical vaccine can take 10 to 20 years for approval from the initial research and development to licensure and commercialization of the product. With parallel tracking now underway for SARS, it is not impossible to see a SARS vaccine commercially available within 5 years or less. To date, no vaccine has been completed from start to finish that quickly but SARS is setting a new precedent for infectious disease vaccines.

Until a SARS vaccine is developed, the quarantine of patients and exposed individuals remain the most effective way of containing this disease. Given the lessons learned from SARS from the outbreaks of 2002/2003, it is expected that future cases of SARS will be limited to sporadic cases, although many countries still lack national pandemic preparedness should a major SARS outbreak occur. There will be continued efforts to work on a SARS vaccine so that countermeasures will be available if and when SARS re-emerges.

APPLICATION OF SARS VACCINE LESSONS TO NEWLY EMERGING INFECTIOUS DISEASES

The global threat of SARS resulted in several groundbreaking vaccine milestones that set the standard for other emerging infectious diseases. Less than 5 months after the first case of SARS was reported, the SARS-CoV genome was first sequenced by the Michael Smith Genome Sciences Centre within 6 days of receiving viral nucleic acid from the British Columbia Centre for Disease Control [9]. Within a few months of the SARS-CoV genome sequencing, animal models for SARS were reported [25, 57, 58] and several SARS vaccine strategies were shown to confer protective immunity upon challenge with the live virus (Table **II**). The experience of SAVI indicate that vaccine development from antigen identification to efficacy testing in animals can be done within a year if research strategies are executed in parallel instead of in sequence [64]. SARS brought attention to the global scientific community that international cooperation and collaboration were important to address infectious diseases that may be of pandemic threat. In addition, SARS highlighted a number of points that were not as clearly evident before the outbreak of SARS. There are few facilities in the world that can manufacture vaccines under cGMP, BSL-3 containment. Certainly, additional manufacturing facilities will be needed for vaccine development for newly emerging infectious diseases, particularly in Canada. This will need to be addressed in the near future in the wake of bioterrorism and other anticipated infectious diseases. Vaccine development is an extremely expensive process and there is a shortage of vaccine companies willing to commit to vaccine development and manufacturing for a vaccine that might not

be widely used. As a result, both the public and private sectors need to work together to ensure a win-win situation for vaccine development [64].

As microbial pathogens are constantly evolving, new pathogens will continue to emerge, particularly from animal reservoirs to cause huge economic loss and high mortality. Already, avian influenza, particularly the H5N1 strain, has returned in 2004 after a seven-year absence. With SARS a threat last year and avian influenza a re-emerging threat in 2004, we can be certain that zoonotic diseases will continue to emerge. As a consequence, scientists will be asked again to provide rapid response solutions. Careful planning for future pandemic threats and development of an effective disease warning system are necessary to handle new and re-emerging infectious diseases.

CONCLUSION

The development of vaccines for SARS-CoV has occurred with astonishing speed in light of new technologies that have become available over the last decade. Past experiences with veterinary coronavirus vaccines indicate that the likelihood of a successful SARS vaccine in humans is high. The main question is whether a SARS vaccine will be developed in time before another SARS epidemic emerges in the human population. Much scientific knowledge has been accumulated on the SARS-CoV, which, in turn have contributed to the rapid development of SARS vaccines. The lessons learned from our experience with SARS will be extremely valuable in demonstrating how science can be applied to other newly emerging and re-emerging infectious diseases.

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