Severe Acute Respiratory Syndrome

Frederick J. Cassels, Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

Background

In the spring of 2003, the world first learned of an outbreak of a newly recognized atypical pneumonia that was subsequently named severe acute respiratory syndrome (SARS). Believed to have originated in the Guangdong province of China in late 2002, SARS quickly spread to Hong Kong, Taiwan, Singapore, Canada, Vietnam, and, ultimately, to a total of 29 countries. Overall, the World Health Organization reported 8,096 probable cases of SARS and 774 fatalities in less than 1 year; 27 of those cases were in the United States [1].

The speed with which the global health community responded to SARS was unparalleled. Shortly after SARS first emerged, the disease's etiological agent was identified as a novel coronavirus called SARS–CoV, which was determined to be phylogenetically distinct from previously known human and animal coronaviruses [2]. Characterization of the virus indicated that it was a single-stranded, positive-sense RNA virus, with a large genome of 29.7 kilobases.

SARS–CoV was discovered to be primarily transmitted by close contact from person to person via large respiratory droplets. Initial signs of illness included flu-like symptoms, with fever, cough, body aches, and malaise after an incubation period ranging from 3 to 10 days. Most patients developed pneumonia, and more than 60 percent of chest X-rays showed infiltrates. Up to 20 percent of individuals had diarrhea.

Epidemiological investigations showed that SARS disproportionately affected healthcare workers and close contacts of SARS patients, such as family members. Higher mortality was observed in older patients, with more than 50 percent of fatalities occurring in people 65 years of age or older. Children were the least likely to develop the disease [3].

The SARS–CoV outbreak likely originated in a few exotic animals in Guangdong marketplaces. SARS–CoV-like viruses, with 99 percent identity to human strains, were isolated primarily from Himalayan palm civets as well as other marketplace animals. From two independent field studies, another animal species, the Chinese horseshoe bat, was subsequently found to harbor a SARS–CoV-like virus that was 93 percent identical to human SARS–CoV [4, 5]. Because SARS–CoV-like virus was not found in wild or farm-raised palm civets, it is thought that the horseshoe bat may serve as the natural reservoir of the virus, with the civet serving as the intermediate host. Both animals were sold in Chinese wet markets.

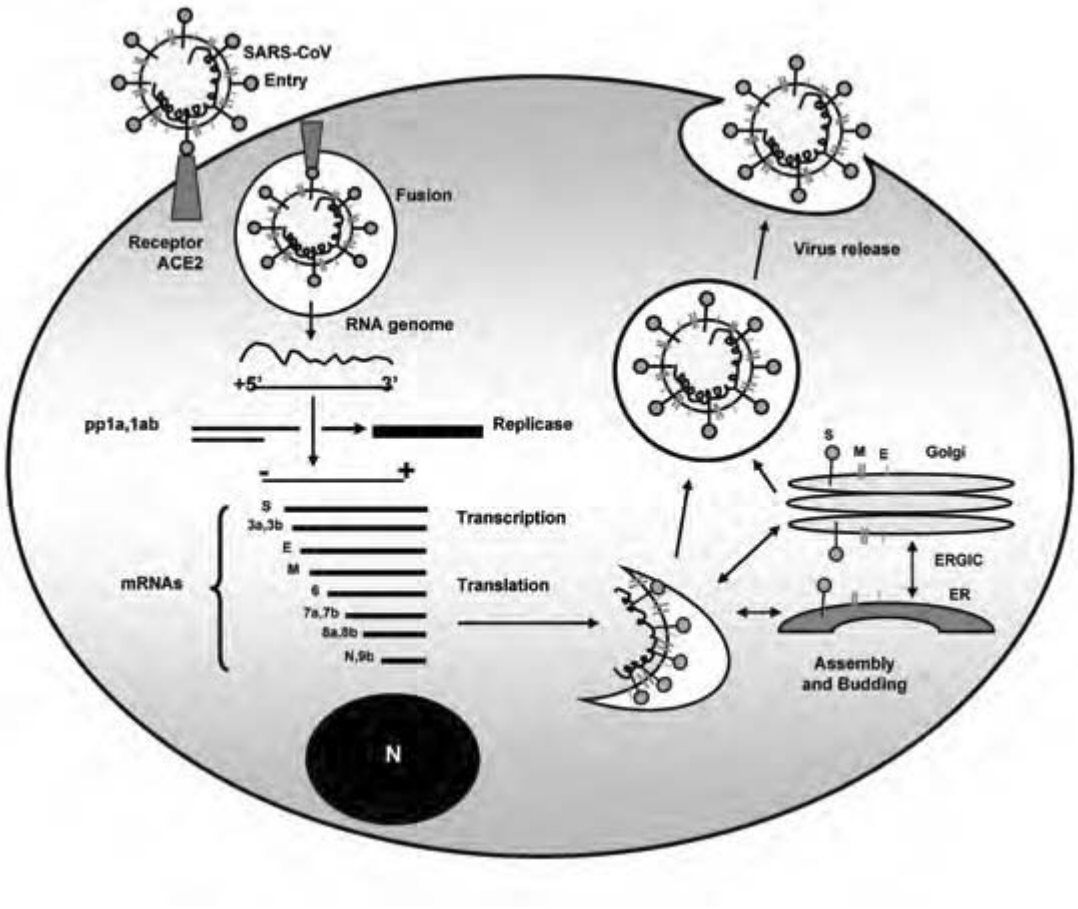
Months after the disease first emerged in mainland China, the clinical syndrome was characterized, the etiological agent was identified, diagnostic tests were developed, and the virus genome was completely sequenced. The speed of scientific understanding and information exchange, combined with critical public health measures such as patient isolation and infection control, eventually led to successful outbreak containment. In July 2003, the World Health Organization officially declared the outbreak over. Since then there have been four separate laboratory-acquired SARS infections—one each in Singapore and Taiwan, and two in China. In addition, two individuals in southern China contracted SARS in December 2003 related to restaurant exposures.

There have been no new SARS cases reported since April 29, 2004. Although the 2003 outbreak has not been repeated, the threat has not disappeared, because an animal reservoir of the precursor virus exists in nature and there is the possibility of an accidental or intentional release of the virus. The population in general, and SARS–CoV researchers specifically, remain at risk without any available prophylactic or therapeutic. Although the global health impact of the SARS 2003–2004 outbreak was tremendous, it paled in comparison to the global economic impact with respect to travel, tourism, and service industries.

SARS Research, Development, and Clinical Testing

National Institute of Allergy and Infectious Diseases (NIAID)-supported scientists have made significant advances in understanding SARS–CoV and its pathogenicity (Figure 1). For example, researchers have identified and characterized the lung receptor molecule, angiotensin converting enzyme-2 (ACE2), to which the S protein adheres [6]. Regions of interaction between the S protein and ACE2 have been mapped and characterized, and the domains of the S protein necessary for viral infection have been determined [7]. This is particularly important in designing improved candidate vaccines and therapeutics. Researchers have learned that the entry of SARS–CoV is blocked by inhibitors of the endosomal protease

FIGURE 1.
SARS–CoV life cycle



SARS–CoV binds to the target cell via interaction between S protein and the cellular receptor ACE2 (angiotensin converting enzyme-2). This complex is translocated to endosomes, S protein is cleaved by cathepsin L, membrane fusion occurs, and the viral genome is released. Viral proteins are transcribed from mRNAs, translated, nucleocapsids assembled in the cytoplasm (from genomic RNA and N protein), then processed through the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). The infected cell releases fully virulent, intact virions through exocytosis [18]. Courtesy of New York Blood Center/Dr. Shibo Jiang

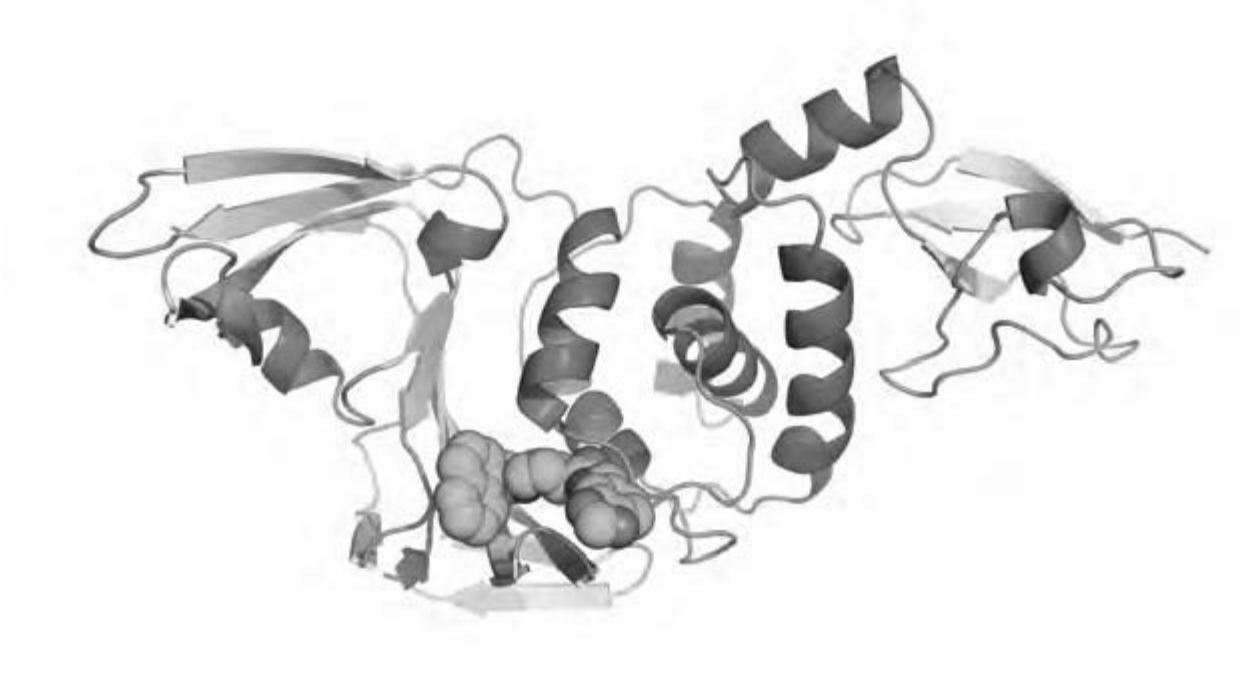
cathepsin L, and a secondary receptor that augments infection, L–SIGN, also was identified and characterized.

Researchers also have discovered that the Papain-like protease (PLpro) of SARS–CoV has deubiquitinating activity, which regulates the location and stability of cellular proteins. They also determined PLpro's three-dimensional structure [8], and this work is contributing to the design of small-molecule inhibitors of this essential enzyme (Figure 2).

Researchers at the Dale and Betty Bumpers Vaccine Research Center, part of NIAID, worked in partnership with Vical, Inc., to manufacture a candidate SARS vaccine that was found to prevent the SARS–CoV from replicating in laboratory mice. The vaccine, composed of a modified piece of DNA that encodes the S protein of SARS–CoV, is expected to stimulate protective immunity in humans. A Phase I open-label clinical study to evaluate safety, tolerability, and immune response to the vaccine was completed in December 2005. The study enrolled 10 healthy volunteers, aged 18 to 50 years, who were given a three-dose vaccine regimen at 1-month intervals. The vaccine was well tolerated, with no or mild systemic or local

FIGURE 2.

PLpro active site with inhibitor



The SARS–CoV papain-like protease (PLpro) enzyme is responsible for proteolytic processing of the viral polyprotein into its functional units. The PLpro active site is depicted in ribbon, and the noncovalent, lead inhibitor in space-filling (sphere) formats [19, 20]. Courtesy of Purdue University/Dr. Andrew D. Mesecar

reactogenicity and no serious adverse events. The vaccine induced neutralizing antibodies, which are strongly associated with recovery from natural SARS infection, and produced cellular immune responses that may be an important component of SARS immunity [9].

Other efforts have been taken by private industry to advance the development of a SARS vaccine. In May 2004, 36 volunteers in Beijing, China, received an inactivated SARS virus vaccine at two dosage levels. The candidate vaccine is produced by a Beijing-based company, Sinovac Biotech Ltd. Most volunteers receiving this vaccine generated an antibody response, and no obvious adverse side effects were noted [10].

Current State of the Science

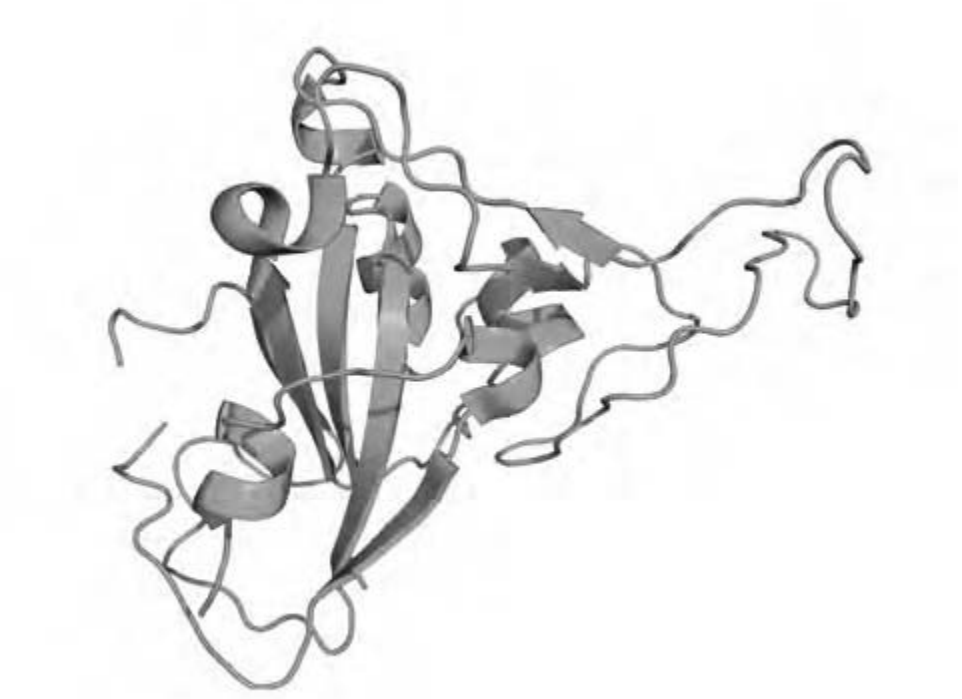
Because it is not known which type of vaccine will be most effective against SARS–CoV, NIAID supports several different approaches to vaccine development.

In 2003, NIAID awarded contracts for the production of experimental inactivated, whole-virus SARS vaccines as well as for the production of a recombinant S protein subunit vaccine [11, 12]. S protein is used by the virus to attach to lung cells. A contract also was awarded to support the generation of a monoclonal antibody to the S protein. This monoclonal antibody demonstrated both prophylactic and therapeutic properties in animals [13]. One of the contractors, Protein Sciences Corporation, has manufactured and released clinical-grade formulations of alum-adjuvanted and unadjuvanted recombinant baculovirus-produced SARS S protein [14]. An Investigational New Drug Application was submitted in mid-2011. The NIAID Vaccine and Treatment Evaluation Units [15] are planning to conduct a Phase I dose-escalation clinical trial of the candidate vaccine in 84 subjects.

In addition, NIAID-supported investigators are pursuing several other vaccine approaches: a soluble S protein SARS vaccine expressed from mammalian cells, an alphavirus replicon vaccine against SARS, and the expression of SARS proteins in virus-like particles. Two alternate strategies being developed are a peptide-based vaccine approach and an attenuated rhabdovirus (rabies) expressing the SARS S protein. As the vaccine development process is long and difficult, it is hoped that multiple strategies will prove safe and effective in animals and, ultimately, in humans.

FIGURE 3.

Receptor Binding Domain crystal structure



Depiction of the X-ray crystal structure of the SARS–CoV S protein receptor binding domain (RDB), amino acids 318–510, in ribbon format. The RDB is a promising subunit vaccine candidate for SARS–CoV [17, 18]. Courtesy of New York Blood Center/Dr. Shibo Jiang

Novel subunit vaccine constructs for an S protein SARS vaccine based on the receptor binding domain (RBD) are being developed by the New York Blood Center (Figure 3). Expression of S protein RBD constructs in 293T and CHO–K1 cells has been demonstrated. All RBD proteins expressed in different expression systems have high specificity and remain in intact conformation, as demonstrated by the binding of a panel of monoclonal antibodies. Recombinant RBD (rRBD) proteins made in various expression systems induce humoral immune responses, as demonstrated by the induction of high titers of antibodies that neutralize live SARS–CoV infection in vaccinated mice [16, 17].

In addition to the vaccine work described, considerable progress has been made on the development of therapeutics for SARS–CoV. Quantitative structure-activity relationship (QSAR) and other computational analysis provided input to further chemical improvement that resulted in a current lead inhibitor with an IC50 (half maximal inhibitory concentration) of 1.6 mM (millimolars) in an enzymatic assay and an EC50 (half maximal effective concentration) of 2.5 mM against the SARS virus in cell culture assays. The development of non-covalent PLpro inhibitors with micromolar antiviral activity appears significant. The crystal structure of PLpro complexed with a lead inhibitor provides a solid foundation for further design development. Investigators demonstrated the synergy in efficacy for 3C-like protease (3CLpro) and PLpro inhibitors, and they are now pursuing parallel discovery and development of therapeutic inhibitors of both the 3CLpro and PLpro enzymatic targets that appear to be most relevant to SARS [18, 19].

Alternative SARS–CoV inhibitors have been investigated based on their ability to block viral entry. Vinyl sulfides identified as very efficient inhibitors include K777, which previously was identified as an inhibitor of *Trypanosoma cruzi*. Second-generation analogs were generated and found to be between twofold and tenfold more potent than K777 and potent against other viruses as well, including Ebola and other human CoVs. Mannose-binding lectin (MBL) can directly inhibit SARS–CoV entry. Using a panel of spike mutants, an *N*-linked glycosylation close to the receptor binding site has been identified as the primary moiety involved in MBL binding, which demonstrated that MBL can inhibit entry only if applied prior to

cathepsin L activation [20]. Unlike several other viral envelopes to which MBL can bind, both recombinant and plasma-derived human MBL directly inhibited SARS–CoV-mediated viral infection. Mutagenesis indicated that a single *N*-linked glycosylation site, N330, was critical for the specific interactions between MBL and SARS–S. Despite the proximity of N330 to the receptor-binding motif of SARS–S, MBL did not affect interactions with the ACE2 receptor or cathepsin L-mediated activation of SARS–S-driven membrane fusion. Thus, binding of MBL to SARS–S may interfere with other early pre- or post-receptor binding events necessary for efficient virus entry [21].

In addition, NIAID contractors have screened 102,000 potential antiviral drugs and other compounds for activity against SARS–CoV. Several compounds have demonstrated antiviral activity and are being further tested in animal models.

Studies also have been conducted on the molecular mechanisms regulating SARS–CoV pathogenesis in young and aged mice. The resulting data suggest that the magnitude and kinetics of a disproportionately strong host innate immune response contributed to severe respiratory distress and lethality. Although the molecular mechanisms governing acute respiratory distress syndrome (ARDS) pathophysiology remain unknown in aged animals, these studies reveal a strategy for dissecting the genetic pathways by which SARS–CoV infection induces changes in the host response, leading to death [22]. The efficacies of candidate vaccines based on a Venezuelan equine encephalitits virus (VEE) attenuated viral replicon particles (VRP) bearing either attenuated (VRP(3014)) or wild-type VEE glycoproteins (VRP(3000)) were compared in young and aged mice. Aged animals receiving VRP(3000)-based vaccines were protected from SARS–CoV disease, while animals receiving the VRP(3014)-based vaccines were not. Because the glycoproteins of VRP(3014) strain differ from those of the wild-type virus by only three amino acids, tools are likely available to elucidate the mechanism of SARS–CoV protection in aged mice [23].

Researchers in NIAID's Laboratory of Infectious Diseases (LID) studied the replication of SARS–CoV in mice, hamsters, and nonhuman primates (NHPs) and established that intranasally administered SARS–CoV replicated efficiently in respiratory tissues. In BALB/c mice and hamsters, the virus replicated to levels that permit an evaluation of vaccines, immunotherapies, and antiviral drugs. In addition, further studies in mice and hamsters demonstrated that primary infection provides protection from re-infection and that antibodies alone can protect against viral replication. This work suggests that vaccines that induce neutralizing antibodies as well as strategies for immunoprophylaxis or immunotherapy are likely to be effective in combating SARS. LID scientists have collaborated with scientists at academic institutions to demonstrate the efficacy of monoclonal antibodies against the spike protein of SARS–CoV in preventing and treating SARS-associated disease in hamsters [13].

The LID investigators observed no clinical illness in young mice, hamsters, or NHPs infected with SARS–CoV. However, because advanced age has been associated with poorer outcome and greater mortality in SARS patients, the NIAID investigators examined whether aged mice might be susceptible to disease. They found that SARS–CoV-infected aged mice demonstrated signs of clinical illness that resolved by day 7 post-infection. The virus-infected aged mice mounted an adaptive immune response to infection; however, in contrast to young mice, they also mounted a proinflammatory cytokine response early post-infection. This work demonstrated in animals an age-related susceptibility to SARS that parallels the human experience [24]. The role of T cells in the pathogenesis and clearance of SARS–CoV was also evaluated in aged mice. Depletion of CD8+ T cells at the time of infection did not affect viral replication or clearance, but depletion of CD4+ T cells resulted in delayed clearance of SARS–CoV from the lungs and was associated with an enhanced immune-mediated interstitial pneumonitis. CD4+ T-cell depletion resulted in reduced neutralizing antibody and cytokine production and reduced pulmonary recruitment of inflammatory cells. Viral clearance in the absence of both CD4+ and CD8+ T cells and antibodies was associated with an innate immune response. These findings provide new insights into the role of CD4+ (but not CD8+ T cells) in primary SARS–CoV infection in this model [25].

The virus-host interactions that governed development of the acute end-stage lung disease cases and deaths from SARS are unknown. LID scientists collaborated with scientists at the University of North Carolina to demonstrate that in mice, SARS–CoV pathogenesis is regulated by a STAT1-dependent but type I, II, and III interferon-independent mechanism. These scientists propose that STAT1 primarily protects mice via its role as an antagonist of unrestrained cell proliferation [26].

The LID scientists also have collaborated with other scientists at the National Institutes of Health, as well as researchers at academic institutions and in industry, to evaluate a number of candidate SARS–CoV vaccines, including inactivated, subunit, vectored, and DNA vaccines, in animal models.

Challenges and Opportunities

The re-emergence of SARS is possible, and the need remains for commercial vaccine and therapeutic development. However, the cost and length of time for product development, and the uncertain future demand, result in unfavorable economic conditions to accomplish this task.

A better understanding of the abilities of and requirements for the SARS virus to infect animals without detrimental effect, and to pass from animal to animal (horseshoe bat to civet) as well as from animal to human, is needed. Findings from this research also could apply to the many other viruses that pass from animals to humans [27].

The potential exists for the exacerbation of disease on exposure to those who have been immunized, as has been seen with respiratory syncytial virus, dengue virus, and feline infectious peritonitis virus [28]. Animal studies suggest that this immunopotentiation may occur with candidate SARS–CoV vaccines that contain the N protein [29].

Improved small- and large-animal models for SARS are needed, particularly those models that better mimic human disease with respect to clinical course and symptoms. Improved animal models will help illuminate the pathophysiology of disease, including innate and adaptive immune responses and immunopotentiation, and help move vaccines and therapeutics through the regulatory and clinical phases and ultimately to licensure [30].

In the development of therapeutics and next-generation vaccines, more work is required to determine the structure/function relationships of critical enzymes and structural proteins. Once these relationships are better understood, improvements to the design of small-molecule and protein inhibitors can occur.

A long-term public health strategy should include both active and passive SARS vaccines as well as therapeutics. This strategy should focus on the impact of the disease on healthcare and service workers and on the elderly, as well as mitigation of economic impact.

As the first pandemic of the 21st century, SARS has provided a unique opportunity for research on the life cycle and components of an emerging or re-emerging disease. Although further research is needed, many recent accomplishments are leading the way toward the development of effective prevention and treatment measures.

REFERENCES

1. World Health Organization. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003 [Global Alert and Response on the Internet]. Geneva: World Health Organization; 2003 Dec 31. Accessed from: www.who.int/csr/sars/country/table2004_04_21/en/index.html
2. Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YS, et al. The genome sequence of the SARS-associated coronavirus. Science. 2003 May 30;300(5624):1399-404. Epub 2003 May 1.
3. World Health Organization. Update 49—SARS case fatality ratio, incubation period [Global Alert and Response on the Internet]. Geneva: World Health Organization; 2003 May 7. Accessed from: www.who.int/csr/sarsarchive/2003_05_07a/en/
4. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, et al. Bats are natural reservoirs of SARS-like coronaviruses. Science. 2005 Oct 28;310(5748):676-9. Epub 2005 Sep 29.
5. Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, Wong BH, et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci U S A. 2005 Sep 27;102(39):14040-5. Epub 2005 Sep 16.
6. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
7. Li W, Wong SK, Li F, Kuhn JH, Huang IC, Choe H, et al. Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. J Virol. 2006 May;80(9):4211-9.
8. Ratia K, Saikatendu KS, Santarsiero BD, Barretto N, Baker SC, Stevens RC, et al. Severe acute respiratory syndrome coronavirus papain-like protease: structure of a viral deubiquitinating enzyme. Proc Natl Acad Sci U S A. 2006 Apr 11;103(15):5717-22. Epub 2006 Mar 31.
9. Martin JE, Louder MK, Holman LA, Gordon IJ, Enama ME, Larkin BD, et al; VRC 301 Study Team. A SARS DNA vaccine induces neutralizing antibody and cellular immune responses in healthy adults in a Phase I clinical trial. Vaccine. 2008 Nov 25;26(50):6338-43. Epub 2008 Sep 26.
10. Lin JT, Zhang JS, Su N, Xu JG, Wang N, Chen JT, et al. Safety and immunogenicity from a Phase I trial of inactivated severe acute respiratory syndrome coronavirus vaccine. Antivir Ther. 2007;12(7):1107-13.
11. Spruth M, Kistner O, Savidis-Dacho H, Hitter E, Crowe B, Gerencer M, et al. A double-inactivated whole virus candidate SARS coronavirus vaccine stimulates neutralising and protective antibody responses. Vaccine. 2006 Jan 30;24(5):652-61. Epub 2005 Aug 26.
12. Kusters I, Matthews J, Saluzzo JF. Manufacturing vaccines for an emerging viral infection—specific issues associated with the development of a prototype SARS vaccine. In: Barrett A, Stanberry L, editors. Vaccines for biodefense and emerging and neglected diseases. London: Academic Press; 2009. p. 148-57.
13. Roberts A, Thomas WD, Guarner J, Lamirande EW, Babcock GJ, Greenough TC, et al. Therapy with a severe acute respiratory syndrome-associated coronavirus-neutralizing human monoclonal antibody reduces disease severity and viral burden in golden Syrian hamsters. J Infect Dis. 2006 Mar 1;193(5):685-92. Epub 2006 Jan 27.

14. Zhou Z, Post P, Chubet R, Holtz K, McPherson C, Petric M, et al. A recombinant baculovirus-expressed S glycoprotein vaccine elicits high titers of SARS-associated coronavirus (SARS–CoV) neutralizing antibodies in mice. Vaccine. 2006 Apr 24;24(17):3624-31. Epub 2006 Feb 9.

15. National Institutes of Allergy and Infectious Diseases. Vaccine and treatment evaluation units (VTEUs) [Internet]. Bethesda (MD): National Institutes of Allergy and Infectious Diseases; c2010. Accessed from: www.niaid.nih.gov/about/organization/dmid/clinical/vteu/Pages/default.aspx

16. Du L, Zhao G, Chan CC, Sun S, Chen M, Liu Z, et al. Recombinant receptor-binding domain of SARS–CoV spike protein expressed in mammalian, insect and *E. coli* cells elicits potent neutralizing antibody and protective immunity. Virology. 2009 Oct 10;393(1):144-50. Epub 2009 Aug 15.

17. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS–CoV—a target for vaccine and therapeutic development. Nat Rev Microbiol. 2009 Mar;7(3):226-36. Epub 2009 Feb 9.

18. Ratia K, Pegan S, Takayama J, Sleeman K, Coughlin M, Baliji S, et al. A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. Proc Natl Acad Sci U S A. 2008 Oct 21;105(42):16119-24. Epub 2008 Oct 13.

19. Ghosh AK, Takayama J, Aubin Y, Ratia K, Chaudhuri R, Baez Y, et al. Structure-based design, synthesis, and biological evaluation of a series of novel and reversible inhibitors for the severe acute respiratory syndrome-coronavirus papain-like protease. J Med Chem. 2009 Aug 27;52(16):5228-40.

20. Glowacka I, Bertram S, Herzog P, Pfefferle S, Steffen I, Muench MO, et al. Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63. J Virol. 2010 Jan;84(2):1198-205. Epub 2009 Oct 28.

21. Zhou Y, Lu K, Pfefferle S, Bertram S, Glowacka I, Drosten C, et al. A single asparagine-linked glycosylation site of the severe acute respiratory syndrome coronavirus spike glycoprotein facilitates inhibition by mannose-binding lectin through multiple mechanisms. J Virol. 2010 Sep;84(17):8753-64. Epub 2010 Jun 23.

22. Rockx B, Baas T, Zornetzer GA, Haagmans B, Sheahan T, Frieman M, et al. Early upregulation of acute respiratory distress syndrome-associated cytokines promotes lethal disease in an aged-mouse model of severe acute respiratory syndrome coronavirus infection. J Virol. 2009 Jul;83(14):7062-74. Epub 2009 May 6.

23. Sheahan T, Whitmore A, Long K, Ferris M, Rockx B, Funkhouser W, et al. Successful vaccination strategies that protect aged mice from lethal challenge from influenza virus and heterologous severe acute respiratory syndrome coronavirus. J Virol. 2011 Jan;85(1):217-30. Epub 2010 Oct 27.

24. Roberts A, Vogel L, Guarner J, Hayes N, Murphy B, Zaki S, et al. Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters. J Virol. 2005 Jan;79(1):503-11.

25. Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, et al. Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS–CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS–CoV infection. J Virol. 2010 Feb;84(3):1289-301. Epub 2009 Nov 11.

26. Frieman MB, Chen J, Morrison TE, Whitmore A, Funkhouser W, Ward JM, et al. SARS–CoV pathogenesis is regulated by a STAT1 dependent but a type I, II and III interferon receptor independent mechanism. PLoS Pathog. 2010 Apr 8;6(4):e1000849.

27. Graham RL, Baric RS. Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. J Virol. 2010 Apr;84(7):3134-46. Epub 2009 Nov 11.

28. Perlman S, Dandekar AA. Immunopathogenesis of coronavirus infections: implications for SARS. Nat Rev Immunol. 2005 Dec;5(12):917-27.

29. Deming D, Sheahan T, Heise M, Yount B, Davis N, Sims A, et al. Vaccine efficacy in senescent mice challenged with recombinant SARS–CoV bearing epidemic and zoonotic spike variants. PLoS Med. 2006 Dec;3(12):e525.

30. Subbarao K, Roberts A. Is there an ideal animal model for SARS? Trends Microbiol. 2006 Jul;14(7):299-303. Epub 2006 Jun 8.