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Severe acute respiratory syndrome (SARS) vaccines

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Introduction

Severe acute respiratory syndrome (SARS) emerged in Guandong Province, southern China, in November 2002. Although several infectious agents, including chlamydia, influenza A subtype H5N1 and human metapneumovirus were considered as a possible cause of SARS, three groups independently reported the isolation of a not previously discovered coronavirus (CoV) from clinical specimens of SARS patients.[1–3] Through electron microscopy, serology and reverse-transcription PCR with consensus- and random-primers and subsequent sequencing of the replicase gene, its identity could be revealed. This virus was consistently found in clinical specimens from patients with the disease and not in healthy controls. To conclusively establish a causal role for this CoV, cynomolgous macaques were inoculated with a SARS-CoV isolate. Because the disease in macaques caused by SARS-CoV infection was pathologically similar to that seen in human patients with SARS, and since the virus was successfully re-isolated from the nasal swabs (Fig. 56–1) and lung lesions of these animals, and since a specific antibody response to the virus was shown in the infected animals, SARS-CoV proved to be the causative agent of this infectious disease.[4–5]

Background

Clinical description

The clinical symptoms of SARS-CoV infection are those of lower respiratory tract disease. Besides fever, malaise and peripheral T-cell lymphocytopenia, affected individuals have slightly decreased platelet counts, prolonged coagulation profiles and mildly elevated serum hepatic enzymes.[6–7] Chest radiography reveals infiltrates with subpleural consolidation or 'ground glass' changes compatible with viral pneumonitis.

The major sources of transmission in humans are droplets that deposit on the respiratory epithelium. Unlike the situation in several other respiratory viral infections, viral load of SARS-CoV in the upper respiratory tract increases progressively to peak at around day 10 after disease onset.[8] Therefore, virus transmission is lower in the first days of illness, a finding supported by epidemiological observations. Overall, if superspreading events are not taken into account, transmissibility of SARS-CoV as indicated by the reproductive number (R_0) has been estimated to be relatively low (2–3).[9,10]

Although the main clinical symptoms are those of severe respiratory illness, SARS-CoV actually also causes a gastrointestinal and urinary tract infection; SARS-CoV can be detected in the feces and urine of patients and electron microscopic studies of biopsies of the upper and lower intestinal mucosae of patients with SARS confirmed the presence of the virus in these tissues[6]. Fecal transmission proved to be important in at least one major community outbreak in Hong Kong (Amoy Gardens), in which over 300 patients were infected within a few days.

Complications

Around 20–30% of individuals with SARS required management in intensive care units and the overall fatality rate reached approximately 10%. In typical cases, which were largely confined to adult and eldery individuals, SARS presented with acute respiratory distress syndrome (ARDS), characterized by the presence of diffuse alveolar damage (DAD) and multi organ dysfunction upon autopsy.[11] The pathological changes in lung alveoli most likely follow a common pathway characterized by an acute phase of protein-rich alveolar fluid influx into the alveolar lumina as a consequence of the injury to the alveolar wall. Subsequently type-2 pneumocyte hyperplasia takes place to replace the loss of infected type-1 pneumocytes and to cover the denuded epithelial basement membrane, resulting in restoration of the normal alveolar architecture. Severe alveolar injury may lead to fibrosis with loss of alveolar function in more protracted cases.

Virology

SARS-CoV is a positive stranded RNA virus, related to coronaviruses from group 2 (Fig. 56–2) despite the fact that it does not encode a hemagglutinin-esterase protein.[12] The genome is packaged together with the nucleocapsid protein, at least three membrane proteins (M, E and ORF3a) and the spike protein. The S1 region within the spike protein and more specifically a 193-amino acid fragment of the S protein (corresponding to residues 318–510) has been identified as the region that interacts with the viral receptor, angiotensin-converting enzyme 2 (ACE2).[13] After engagement with ACE2, SARS-CoV fuses with host cell membranes by a fusion mechanism similar to that exerted by class I fusion proteins. The conformational changes of the two heptad regions located in the S2 region, HR-1 and HR-2, cause the formation of an oligomeric structure, leading to fusion between the viral and target-cell membranes. The

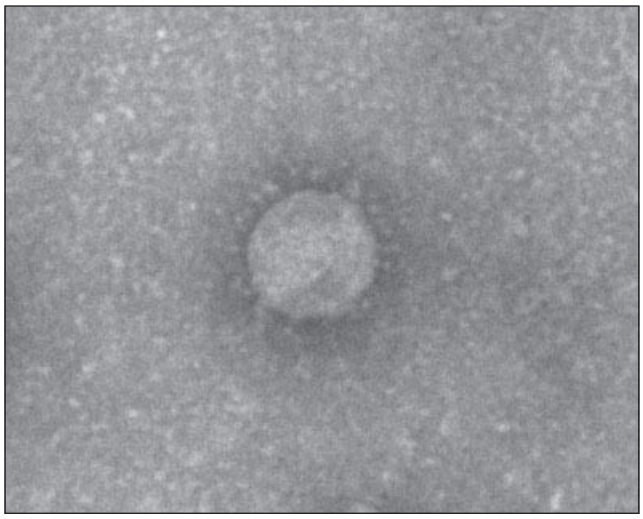

Figure 56–1 Negative-contrast electron microscopy of a SARS-CoV particle isolated from the nasal swabs of SARS-CoV infected macaques.

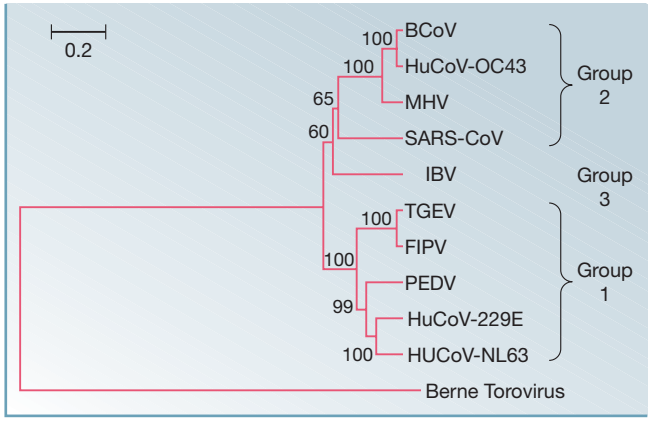

Figure 56–2 Phylogenetic tree based on deduced amino acid sequences of the coronavirus replicase ORF1b gene for bovine coronavirus (BCoV), human coronavirus 22E (HuCoV-OC43), mouse hepatitis virus (MHV), SARS-CoV, infectious bronchitis virus (IBV), transmissible gastroenteritis virus (TGEV), feline infectious peritonitis virus (FIPV), porcine epidemic diarrhea virus (PEDV), human coronavirus 229E (HuCoV-229E), human coronavirus NL63 (HuCoV-NL63) and Berne Torovirus (used as an outgroup).

genome also encodes two large poly-proteins with diverse enzymatic activities needed for efficient replication and several accessory proteins with unknown function.

Pathogenesis as it relates to prevention

Three important features of the SARS pathogenesis may be relevant for intervention strategies. First, progressive age dependence in mortality and disease severity is observed in SARS patients.[14] In fact, none of the SARS-CoV infected children aged below 12-years in Hong Kong required intensive care or mechanical ventilation.[15] This is not totally explained by co-morbid factors but similar age dependence in mortality is seen in patients with other (non-viral) causes of acute respiratory stress syndrome.[16] Secondly, virus transmission is low in the first days of illness and peaks around day 10 after disease onset.[18] Finally, several studies revealed that high viral load in the nasopharyngeal aspirate was found to be an independent predictor of mortality.[17–18] Therefore, vaccine strategies aimed at reducing the viral load may suffice to provide clinical benefit.

Diagnosis

Although seroconversion usually occurred in weeks 2 or 3 of illness, serodiagnosis represents the gold standard for confirmation of a SARS diagnosis. Real-time PCR assays, however, usually detect SARS-CoV during the first week in specimens of the lower respiratory tract (e.g., bronchoalveolar lavage, sputum, endotracheal aspirates), nasopharyngeal aspirate, throat swabs and/or serum.[19] Fecal samples may show very high viral loads toward the end of the first week and second week of illness. More recently developed assays are able to detect SARS-CoV nucleocapsid protein in serum only few days after onset of disease.[20]

Treatment and prevention with antimicrobials

The first efforts to treat SARS patients were mainly based on the use of ribavirin and corticosteroids. Ribavirin, which targets IMP dehydrogenase, has been known a long time as a broad-spectrum antiviral agent. However, current data do not support the use of ribavirin for SARS treatment; in vitro studies did not show significant antiviral activity[21] and ribavirin enhanced the infectivity of SARS-CoV in mice.[22] On the other hand, a protective effect of interferon (IFN)-α has been obtained in a preliminary study during the SARS outbreak.[23] These results are in concordance with several studies that noted antiviral activity in vitro[21,24] and animal studies showing that pegylated IFN-α effectively reduced SARS-CoV replication and excretion, viral antigen expression by type 1 pneumocytes and the pulmonary damage in cynomolgous macaques that were infected experimentally with SARS-CoV.[25] Because IFNs are used clinically to treat viral infections, these drugs could be considered for off label use in SARS prophylactic or early-postexposure treatment of SARS should it re-emerge.

Epidemiology

Reservoirs of infection

Because many of the early SARS patients in Guandong had epidemiological links to the live-animal market trade, different animal species were tested for the presence of SARS like viruses. A SARS-like coronavirus, which had more than 99% homology with human SARS-CoV, was detected by RT-PCR in the nasal and fecal swabs of palm civets (*Paguma larvata*) and a raccoon dog (*Nyctereutes procyonoides*).[26] More recently, bats have been shown act as natural reservoirs for SARS-like CoVs.[27,28] However, sequence comparison of the spike genes from bat SARS-like CoV and palm civet SARS-like CoV revealed only 64% genetic homology. Subsequent studies by Tang et al, have demonstrated that approximately 6% of bats sampled in China were positive for CoVs.[29] Interestingly, these CoVs are genetically diverse and many bat CoVs clustered with existing group 1 viruses, while others formed a separate lineage that included only viruses from bats (putative group 5). Other SARS-CoV like viruses clustered in a putative group 4 consisting of two subgroups, one of bat CoVs and another of SARS-CoVs from humans and other mammalian hosts. Although the direct progenitor of the SARS-CoV isolated from palm civets has not been determined, bats are the most likely reservoir of SARS-CoV that infected those animals. Subsequent major genetic variations in the spike gene of the viruses from civet cats seem to have been essential for the transition from animal to human transmission to human to human transmission that eventually caused the SARS outbreak of 2002–2003.

Overall, a wide range of animal species, including rodents (mice and hamsters),[30,31] carnivores (ferrets and cats)[32] and

non-human primates (cynomolgus- and rhesus-macaques, common marmosets and African green monkeys)[4,33,34] can be experimentally infected with SARS-CoV. Most species show no clinical signs of disease, although the virus replicates efficiently in respiratory tissues. Aged mice and ferrets on the other hand, show signs of clinical disease, albeit in the absence of the typical lung lesions seen in humans with SARS.[35] In contrast, SARS-CoV inoculation in the respiratory tract of cynomolgus macaques causes infection of bronchial epithelial cells and type-1 pneumocytes 1–4 days post infection followed by extensive type-2 pneumocyte hyperplasia in the lungs at 4–6 days post infection. The lesions, consisting of multiple foci of acute DAD and characterized by flooding of alveoli with protein-rich oedema fluid mixed with variable numbers of neutrophils, are quite similar to those observed in humans in the acute stages of SARS.[11]

Risk groups

There is at present no evidence for the virus persisting in the human population. Possible options for the re-emergence of SARS include the re-emergence of the virus from an animal reservoir or the escape of the virus from laboratories, which already occurred on three occasions. The re-emergence of the virus from its animal reservoir remains possible, given that the virus is detectable in the feces and respiratory secretions of some animals. Indeed, SARS-CoV re-emerged in four patients in Guangdong in December 2003, although these SARS-like CoVs caused milder clinical disease.[36]

Passive immunization

In SARS patients that recover, high levels of neutralizing antibody responses are observed, suggesting that antibody responses play a role in determining the ultimate disease outcome of SARS-CoV-infected patients.[37] Although attempts have been made to test the efficacy of serum preparations from seroconvalescent SARS patients in the acute phase of SARS, no conclusive evidence has been obtained regarding their efficacy. In mice, on the other hand, SARS-CoV infection is efficiently controlled upon passive transfer of convalescent immunoglobulines.[30] The concept that antibodies protect against SARS has been further explored through the generation of human monoclonal antibodies against SARS-CoV. Prophylactic administration of a human monoclonal antibody reduced replication of SARS coronavirus in the lungs of infected ferrets by 3 logs, completely prevented the development of SARS coronavirus-induced macroscopic lung pathology, and abolished shedding of virus in pharyngeal secretions.[38] In subsequent studies several other monoclonal antibodies were evaluated for their efficacy in mouse- and hamster-models.[39,40]

Active immunization

Less than a year after the first SARS outbreak a range of candidate vaccines was developed and early 2006 some companies in China and the US initiated phase one trials and several other candidate SARS vaccines are at various stages of pre-clinical and clinical development. Table 56–1 displays an overview of vaccines that have been tested for efficacy in animal models.

Inactivated and subunit SARS vaccines

Inactivated SARS vaccines have been reported to elicit high titers of spike-specific neutralizing antibodies. Few studies, however, have addressed whether inactivated whole SARS-CoV virions confer protection from virus challenge. Mice that were immunized twice with a candidate SARS-CoV vaccine, produced through a two-step inactivation procedure involving sequential formaldehyde and U.V. inactivation, developed high antibody titres against the SARS-CoV spike protein and high levels of neutralizing antibodies.[41] Moreover, the vaccine conferred protective immunity as demonstrated by prevention of SARS-CoV replication in the respiratory tract of mice after intranasal challenge with SARS-CoV. Protection of mice was correlated to antibody titer against the SARS-CoV S protein and neutralizing antibody titer.

Similar results have been obtained using a beta-propiolactone inactivated SARS-CoV vaccine in mice.[42] In addition, two Chinese groups have demonstrated protective efficacy of inactivated SARS vaccines in rhesus monkeys.[43,44] A soluble recombinant polypeptide containing the N-terminal segment of the spike glycoprotein may suffice to induce neutralizing antibodies and protective immunity in mice.[45]

DNA vaccines

A DNA vaccine encoding the spike glycoprotein of the SARS-CoV induces T cell and neutralizing antibody responses, as well as protective immunity, in a mouse model.[46] Moreover, antibody responses in mice vaccinated with an expression vector encoding a form of S that includes its transmembrane domain

Table 56–1 SARS-CoV Vaccines[a]

Vaccine	Animal Species	Immunogenicity[b]	Protection	References
Inactivated whole virus	Mice and macaques	Neutralizing Abs	Yes	41-44
Subunit	Mice	Neutralizing Abs	Yes	45
DNA vaccines	Mice	Neutralizing Abs	Yes	46, 47
Adenovirus vector	Macaques and mice	Neutralizing Abs	Yes	48, 49
Vaccinia virus vector	Mice Macaques ferrets	Neutralizing Abs Neutralizing Abs No neutralizing antibodies	Yes Yes No	50 51 52
Parainfluenza virus vector	Macaques and hamsters	Neutralizing Abs	Yes	53, 54
Rhabdovirus vector	Mice	Neutralizing Abs	Yes	55,56

[a]only those SARS-CoV vaccines containing the spike protein/gene and tested for protection against a SARS-CoV challenge are listed.
[b]presence of neutralizing antibodies at time of challenge.

elicited neutralizing antibodies. Viral replication was reduced by more than six orders of magnitude in the lungs of mice vaccinated with these S plasmid DNA expression vectors, and protection was mediated by a humoral but not a T-cell-dependent immune mechanism. Subsequent studies using a prime-boost combination of DNA and whole killed SARS-CoV vaccines elicited higher antibody responses than DNA or whole killed virus vaccines alone.[47] Apart from this study, several other groups have analysed the immunogenicity of SARS DNA vaccines but none of these challenged the vaccinated animals with SARS-CoV.

Adenovirus-based vaccines

Adenovirus-vector based vaccination strategies against SARS-CoV were employed early on after the SARS outbreak to demonstrate that vaccinated rhesus macaques developed virus-neutralizing antibody responses against fragment S1 of spike and T-cell responses against the nucleocapsid.[48] More recently, See et al[49] demonstrated that vaccination of C57B1/6 mice with adenovirus type 5-expressing spike and nucleocapsid administered intranasally, but not intramuscularly, significantly limited SARS-CoV replication in the lungs.

Vaccinia virus-based vectors

The highly attenuated modified vaccinia virus Ankara (MVA) has been used to express the spike glycoprotein of SARS-CoV in vaccination experiments using mouse, ferret and rhesus monkey models.[50–52] Intranasal and intramuscular administration of MVA encoding the SARS-CoV spike protein led to the induction of a humoral immune response in BALB/c mice, as well as reduced viral titers in the respiratory tract.[45] Similary, protective responses were induced in rhesus monkeys.[46] However, in one study in ferrets, vaccination with MVA encoding the spike induced only moderate antibody responses and consequently did not protect against intranasal SARS-CoV infection but resulted in an inflammatory response in the livers of the vaccinated ferrets.[52] Whether these aberrant responses resulted from immunopathological mechanisms, such as antibody dependent enhancement of infection or represented recall responses to viral antigen in the liver is not clear at the moment and deserves further investigation.

Mucosal vaccines

Recombinant bovine-human parainfluenza virus type 3 vector (BHPIV3) is being developed as a live attenuated, intranasal pediatric vaccine against human parainfluenza virus type 3. Immunization of African green monkeys with a single dose of BHPIV3 expressing SARS-CoV spike protein administered via the respiratory tract induced the production of SARS-CoV neutralizing antibodies.[53] A recombinant BHPIV3 expressing SARS-CoV structural protein (S, M and N) individually or in combination has been evaluated for immunogenicity and protective efficacy in hamsters.[54] In the absence of spike, expression of M, N or E did not induce a detectable serum SARS-CoV-neutralizing antibody response and no protection against SARS-CoV challenge in the respiratory tract, whereas the vectors expressing the S protein induced neutralizing antibody responses and protection.

Other vector-based vaccines

Recombinant rabies virus expressing the S or the N protein of SARS-CoV induced a neutralizing antibody response in mice.[55] Similarly an attenuated vesicular stomatitis virus vector that encodes the SARS-CoV spike may be used to induce neutralizing antibody responses.[56] Mice vaccinated with this vesicular stomatitis virus developed SARS-CoV-neutralizing antibodies and were able to control a challenge with SARS-CoV performed at 1 month or 4 months after a single vaccination. In addition, by passive antibody transfer experiments, those authors demonstrated that the antibody response induced by the vaccine was sufficient for controlling SARS-CoV infection.

Immunogenicity of vaccine

Antibody and cellular responses

The importance of assessing immunogenicity of candidate SARS-CoV vaccines using VN assays is well acknowledged, but the variety of VN tests in use is a significant problem since there is at this time no consensus on the most sensitive, specific and reproducible assay system. To compare data from each of the candidate vaccines requires international standardization of the immunological assays and the availability of an antibody standard used for the evaluation of these vaccines. To test cross reactivity of antibodies generated by vaccination, murine leukemia virus was used to generate infectious particles containing different S proteins.[57] The importance of cell-mediated immunity in vaccine induced protection may be limited.

Correlates of protection

So far, work in animal models shows that neutralizing antibodies alone are effective for prevention and treatment of SARS. Thus, mice immunized with inactivated virus vaccines, live-recombinant vaccines expressing the SARS-CoV spike protein, using rabies virus, vesicular stomatitis virus, bovine parainfluenza virus type 3, adenovirus or attenuated vaccinia virus MVA as a vector, as well as mice immunized with DNA vaccines expressing the spike gene, developed neutralizing antibodies to SARS-CoV and were protected against SARS-CoV challenge. Studies using monoclonal antibodies directed against different regions of the spike protein (S1 and S2) have demonstatrated potent neutralization of SARS-CoV in vitro.[58–59] Conversely, peptides which are located in these regions were able to induce neutralizing antibodies.[45,60,61]

More recent studies by He et al[62] have shown that the major neutralizing epitopes of SARS-CoV have been maintained during cross-species transmission, suggesting that receptor binding domain-based vaccines may induce broad protection against both human and animal SARS-CoV variants.

Although not all correlates of protection from SARS have been identified in human SARS-CoV infections, neutralizing antibodies are present in convalescent human serum. However, the neutralizing antibody titer necessary to achieve protection in humans exposed to SARS-CoV is still unknown. Despite the fact that long-lived memory T cell responses against SARS-CoV nucleocapsid and spike protein have been demonstrated in recovered SARS patients their relevance in antiviral protection is not well understood.[63,64]

Safety (adverse events)

Enhanced disease and mortality have been observed in kittens immunized against or infected with a type-I coronavirus, feline infectious peritonitis virus (FIPV), when subsequently exposed

to FIPV infection.[65] Macrophages are able to take up feline coronavirus-antibody complexes more efficiently causing the virus to replicate to higher titers. Interestingly, one study also demonstrated that antibodies against human SARS-CoV isolates enhance entry of pseudo-typed viruses expressing the civet cat SARS-like CoV-spike protein into cells but not replication.[66] However, so far there is no evidence for enhanced replication following SARS-CoV challenge in previously immunized animals.

One other problem which may arise after vaccination with whole inactivated virus when absorbed with certain adjuvants such as alum, could relate to the induction of skewed Th2 recall responses similar to what has been observed in children vaccinated with inactivated respiratory syncytial and measles virus vaccines.

Future vaccines

Although much effort has been focused on developing a SARS vaccine, the commercial viability of developing a vaccine for SARS-CoV will ultimately depend on whether the virus re-emerges in the near future. It is questionable whether possible future outbreaks will be major, but vaccines, antivirals or passive immunization would be relevant in the context of protecting high-risk individuals such as laboratory and health care workers. Alternatively, future vaccines may be generated from the full-length infectious cDNA clone of SARS-CoV[67] once viral virulence factors are understood and attenuated strains obtained through manipulation of the SARS-CoV genome.

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