CHAPTER

36

SARS

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OUTLINE

Introduction

Etiologic Agent

Classification and Antigens Encoded

Epidemiology

Significance as Public Health Problem and Potential as Biothreat Agent

Clinical Disease

Treatment

Pathogenesis

Innate Immune Response to Infection

Humoral Immune Response

Cellular Immune Response

Vaccines
Inactivated whole virus and subunit vaccines
Vectored vaccines

Basic Science and Rationale of New Generation Vaccines

Preclinical Development, Including Relevant Animal Models

Clinical Trials

Postexposure Immunoprophylaxis

Prospects for the Future

Key Issues

ABSTRACT

Five years after the first severe acute respiratory syndrome (SARS) outbreak, several candidate SARS-coronavirus (CoV) vaccines are at various stages of preclinical and clinical development. Based on the observation that SARS-CoV infection is efficiently controlled upon passive transfer of antibodies directed against the spike (S) protein of SARS-CoV, vaccines containing the S protein have been formulated. Animals immunized with inactivated whole virus vaccines or live-recombinant vaccines expressing the SARS-CoV S protein (e.g., 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 S protein gene all developed neutralizing antibodies to SARS-CoV and were protected against SARS-CoV challenge.

Although much effort has been focused on developing a SARS vaccine, the commercial viability of such a vaccine for SARS-CoV will ultimately depend on whether the virus re-emerges in the near future. This vaccine

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should induce highly cross-reactive neutralizing antibodies to protect against newly emerging viruses related to SARS-CoV and protect both the gastrointestinal and respiratory tract in the absence of significant side effects. Given the fact that in the previous outbreak mainly the elderly succumbed to the infection, special attention should be given to vaccines that are able to efficiently protect aged individuals.

INTRODUCTION

Severe acute respiratory syndrome coronavirus (SARS-CoV) first emerged in the human population in November 2002. Phylogenetic analysis of SARS-CoV isolates from animals indicated that this virus most probably originated from bats, was transmitted first to palm civets and subsequently to humans at the wet markets in southern China. Subsequent outbreaks occurred early 2003 in Hong Kong, Hanoi, Toronto, and Singapore, and could be directly traced back to one index patient who acquired the infection in Guangdong and traveled to Hong Kong. A worldwide epidemic was halted through the efforts of the World Health Organization, which responded rapidly to this threat by issuing a global alert, rigorous local containment efforts, warning against unnecessary travel to affected areas, and by creating a network of international experts to combat this virus. In the end only 8096 people became ill, and 774 people died in this first SARS epidemic. Because SARS-CoV could re-emerge and cause another epidemic at any time, development of effective vaccines remains of vital importance.

ETIOLOGIC AGENT

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 previously unrecognized CoV from clinical specimens of SARS patients (Peiris et al., 2003; Rota et al., 2003; Drosten et al., 2003).

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 and consistently demonstrated in clinical specimens from patients with the disease but 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 and lung lesions of these animals, and 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 (Fouchier et al., 2003; Kuiken et al., 2003).

CLASSIFICATION AND ANTIGENS ENCODED

SARS-CoV is a single-stranded, positive-sense RNA virus, phylogentically related to coronaviruses from group 2 despite the fact that it does not encode a hemagglutinin-esterase protein (Snijder et al., 2003). The genome is packaged together with the nucleocapsid protein, at least five membrane proteins (M, E, 3a, 7a, and 7b) and the spike (S) protein (Fig. 36.1). The S1 region within the S 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 cell receptor, angiotensin-converting enzyme 2 (Li et al., 2005a). The majority of neutralizing antibodies are directed against this region of the S protein. Antibodies raised against the N-terminal region of 3a protein or the M

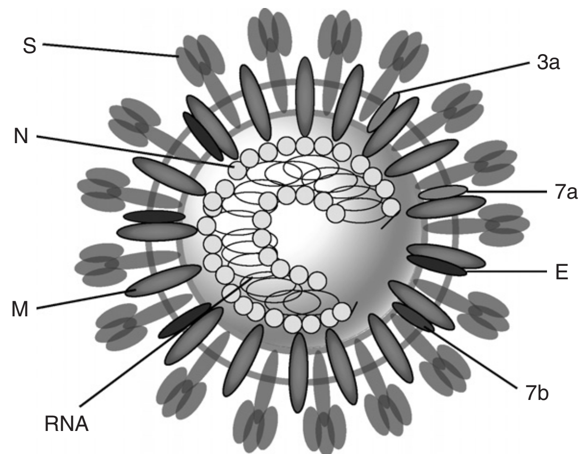

FIGURE 36.1 Schematic diagram of the SARS-CoV particle. S, spike protein; M, membrane protein, E, envelope protein; N, nucleocapsid protein; 3a, 7a, and 7b; structural proteins of SARS-CoV.

protein also inhibit SARS-CoV replication in vitro but their relevance in protection remains unclear. The genome also encodes two large poly-proteins with diverse enzymatic activities needed for efficient replication and several accessory proteins with unknown function (3b, 6, 8a, 8b, and 9b).

EPIDEMIOLOGY

At the end of 2004, 30 countries reported a total of 8096 probable cases of SARS (Fig. 36.2). Pathogenic SARS-CoVs do not circulate in the human population at the moment, but their re-emergence from animal reservoirs may likely occur in the future. 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. Soon after the outbreak, 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*) (Guan et al., 2003). More recent studies indicate that bats may potentially act as natural reservoirs for SARS-like CoVs (Li et al., 2005b; Lau et al., 2005). However, sequence comparison of the S protein genes from bat SARS-like CoV and palm civet SARS-like CoV revealed only 64% genetic homology. Subsequent studies by Tang et al. (2006) have demonstrated that approximately 6% of bats sampled in China were positive for CoVs. 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. However, from these studies the direct progenitor of the SARS-CoV isolated from palm civets has not been identified. Major genetic variations in the S protein gene of these viruses from civet cats, seemed essential for the transition from animal-to-human transmission

FIGURE 36.2 Reported suspected SARS cases from November 1, 2002 to July 31, 2003 (data from the World Health Organization, http://www.who.int/csr/sars/country/table2004_04_21/en/index.html). Countries with >5 suspected cases are indicated.

to human-to-human transmission, which eventually caused the SARS outbreak of 2002–2003.

SIGNIFICANCE AS PUBLIC HEALTH PROBLEM AND POTENTIAL AS BIOTHREAT AGENT

There is at present no evidence for the virus persisting in the human population. Possible options for the re-emergence of SARS include the escape of the virus from laboratories, which has 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 (Liang et al., 2004). The US National Institute of Allergy and Infectious Diseases Biodefense Network classified SARS-CoV as a category C priority pathogen pointing out that SARS-CoV could be a potential biothreat agent.

CLINICAL DISEASE

The clinical symptoms of SARS-CoV infection are those of lower respiratory tract disease and include fever, malaise, peripheral T cell lymphocytopenia, decreased platelet counts, prolonged coagulation profiles, and mildly elevated serum hepatic enzymes (Peiris et al., 2004; Li et al., 2004). Chest radiography reveals infiltrates with subpleural consolidation or “ground glass” changes compatible with viral pneumonitis. Around 20–30% of individuals with SARS require management in intensive care units and the overall case:fatality rate reached approximately 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 (Peiris et al., 2004). 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.

Three features of SARS may be relevant for intervention strategies. First, progressive age dependence in mortality and disease severity is observed in SARS patients. In fact, none of the SARS-CoV-infected children aged below 12 years in Hong Kong required intensive care or mechanical ventilation (Ng et al., 2004). This is not totally explained by comorbid factors but similar age dependence in mortality is seen in patients with other (nonviral) causes of acute respiratory stress syndrome (Rubenfeld et al., 2005). Second, virus transmission is low in the first days of illness and peaks around day 10 after disease onset (Chu et al., 2004). Finally, several studies revealed that high viral load in the nasopharyngeal aspirate was found to be an independent predictor of mortality (Hung et al., 2004; Chu et al., 2004). Therefore, vaccine strategies aimed at reducing the viral load may suffice to provide clinical benefit.

TREATMENT

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 (Cinatl et al., 2003) and ribavirin enhanced the infectivity of SARS-CoV in mice (Barnard et al., 2006). On the other hand, a protective effect of interferon (IFN)-α has been observed in a preliminary study during the SARS outbreak (Loutfy et al., 2003). These results are in concordance with several studies that noted antiviral activity in vitro (Cinatl et al., 2003; Hensley et al., 2004) 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 (Haagmans et al., 2004). However, despite an extensive literature reporting on SARS treatments, it is not possible to determine whether treatments benefited patients during the SARS outbreak. Because of variation in treatment regimens—particularly the wide range in doses, duration of therapy, and route of administration of ribavirin and corticosteroids, no clear conclusion can be drawn regarding the efficacy of the drugs tested (Stockman et al., 2006). In the event of a future outbreak of SARS-CoV or another novel agent, attempts should be made to develop treatment protocols, organize randomized trials and to collect and contribute information for a standardized minimum dataset that could facilitate analysis of treatment outcomes among different settings (Stockman et al., 2006).

PATHOGENESIS

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 peaked around day 10 after disease onset (Peiris et al., 2004). Therefore, virus transmission may be less efficient in the first days of illness, a finding supported by epidemiological observations. Real-time PCR assays 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 (Chan et al., 2004), whereas fecal samples may show very high viral loads toward the end of the first week and second week of illness. In typical cases, which were largely confined to adult and elderly individuals, SARS presented with acute respiratory distress syndrome, characterized by the presence of diffuse alveolar damage and multiorgan dysfunction upon autopsy (Nicholls et al., 2003). 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 (Fig. 36.3).

INNATE IMMUNE RESPONSE TO INFECTION

It has been hypothesized that the pathological changes observed in the lungs are initiated by a disproportional innate immune response, illustrated by elevated levels of inflammatory cytokines and chemokines, such as CXCL10 (IP-10), CCL2 (MCP-1), IL-6, IL-8, IL-12, IL-1β, and IFN-γ (Huang et al., 2005; Wong et al., 2004). These in vivo data have been confirmed in vitro, demonstrating that SARS-CoV infection induces a range of cytokines and chemokines in diverse cell types (Cheung et al., 2005; Law et al., 2005). Although in vitro studies argued that production of type I IFNs is inhibited or delayed by SARS-CoV, in SARS-CoV-infected macaques, and also early during SARS in humans, type I IFNs can be readily demonstrated (De Lang et al., 2007). Because prophylactic treatment of macaques with pegylated IFN-α reduces SARS-CoV replication in the lungs, regulation of the production of IFNs may be important in controlling SARS (Haagmans et al., 2004).

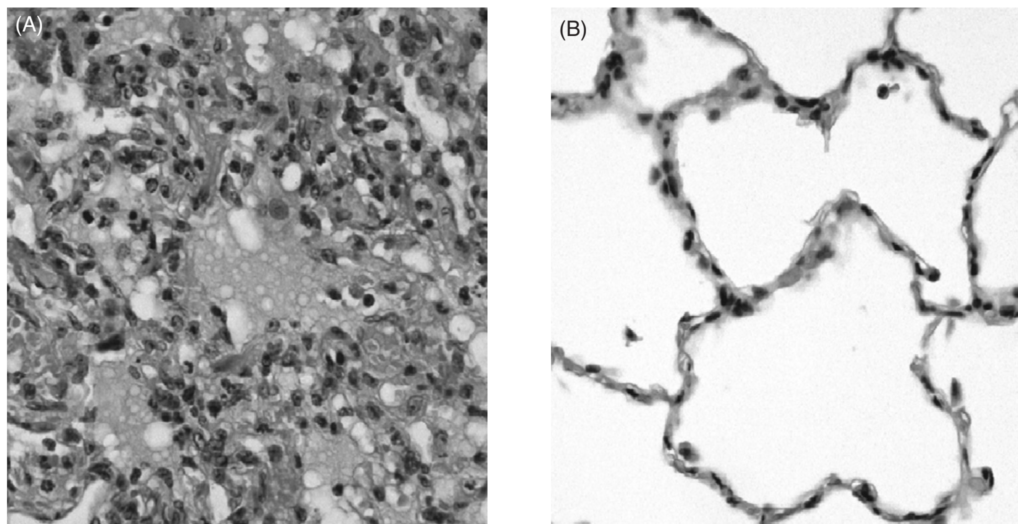

FIGURE 36.3 Acute lung injury after experimental infection of nonhuman primates with SARS-CoV. Shown are the histological changes in the lungs after SARS-CoV infection (A) as compared to the normal lung tissue (B). Viral infection of bronchiolar epithelial cells, type 1 and type 2 pneumocytes results in the influx of macrophages and neutrophils (A). The subsequent loss of type 1 cells may cause flooding of the alveolus by edema fluid (A) and in later stages fibrosis of the alveolar wall (deposition of collagen and influx of fibroblasts).

HUMORAL IMMUNE RESPONSE

Seroconversion usually occurs in weeks 2 or 3 of illness and virus-neutralizing antibodies can be detected in convalescent human serum. In patients who had recovered from SARS-neutralizing antibody titers peaked at month 4 but were undetectable in 16% of patients at month 36 (Cao et al., 2007). Neutralizing antibodies may be directed against different regions of the S protein (S1 and S2) as monoclonal antibodies against these epitopes exert potent neutralization of SARS-CoV in vitro (Sui et al., 2004; Lip et al., 2006). Conversely, peptides which are located in these regions were able to induce neutralizing antibodies (Bisht et al., 2005; Keng et al., 2005; Zhang et al., 2004).

Although experiments in diverse animal models have revealed that relatively low levels of neutralizing antibodies exert potent protection against lower respiratory tract infection, the neutralizing antibody titer necessary to achieve protection in humans exposed to SARS-CoV is not known. A concern in case of reemergence of SARS is the possible absence of cross protection against these viruses. However, recent studies by He et al. (2006) have shown that the some 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.

CELLULAR IMMUNE RESPONSE

In most SARS autopsies, extensive necrosis of the spleen and atrophy of the white pulp with severe lymphocyte depletion have been observed. On the other hand, rapid phase in peripheral lymphocyte recovery usually coincided with improved clinical conditions of SARS patients (Peiris et al., 2004). Long-lived memory T cell responses against SARS-CoV nucleocapsid and S protein have been demonstrated in recovered SARS patients, although their relevance in antiviral protection is not well understood (Yang et al., 2006; Peng et al., 2006; Li et al., 2006). However, despite potent immune responses and clinical recovery, peripheral lymphocyte counts in the recovered patients were not restored to normal levels (Li et al., 2006).

Interestingly, mice that lack NK-T cells, or NK cells, or T and B cells all cleared the virus by day 9 after infection (Glass et al., 2004). These data argue that cell-mediated immune responses are not essential to control virus clearance.

VACCINES

Based on the observation that SARS-CoV infection is efficiently controlled upon passive transfer of antibodies directed against the S protein of SARS-CoV, a range of vaccines containing the S protein/gene has been developed. Animals immunized with inactivated whole virus vaccines or live-recombinant vaccines expressing the SARS-CoV S protein (e.g., 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 S protein gene all developed neutralizing antibodies to SARS-CoV and were protected against SARS-CoV challenge. Table 36.1 displays an overview of vaccines that have been tested for efficacy in animal models.

Inactivated Whole Virus and Subunit Vaccines

Inactivated SARS vaccines have been reported to elicit high titers of S protein-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 titers against the SARS-CoV S protein and high levels of neutralizing antibodies (Spruth et al., 2006) (see Chapter 11). 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 the 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 (Stadler et al., 2005). In addition, two Chinese groups have demonstrated protective efficacy of inactivated SARS vaccines in rhesus monkeys (Qin et al., 2006; Zhou et al., 2005). A soluble recombinant polypeptide containing the N-terminal segment of the S glycoprotein may suffice to induce neutralizing antibodies and protective immunity in mice (Bisht et al., 2005). In addition, a trimeric recombinant S protein was able to elicit an efficacious protective immune response in hamsters (Kam et al., 2007).

One of the most promising vaccine candidates is based on the combination of recombinant S protein with the Protollin adjuvant. In both young and aged mice, an intranasal Protollin-formulated S protein

TABLE 36.1 SARS-CoV vaccines[a]

Author	Vaccine[b]	Animal species	Immunogenicity[c]	Protection
Spruth et al. (2006)	Inactivated whole virus	Mice and macaques	Neutralizing Abs	Yes
Stadler et al. (2005)	Inactivated whole virus	Mice	Neutralizing Abs	Yes
Qin et al. (2006)	Inactivated whole virus	Macaques	Neutralizing Abs	Yes
Zhou et al. (2005)	Inactivated whole virus	Macaques	Neutralizing Abs	Yes
Bisht et al. (2005)	Subunit	Mice	Neutralizing Abs	Yes
Kam et al. (2007)	Subunit	Mice	Neutralizing Abs	Yes
Hu et al. (2007)	Subunit	Mice	Neutralizing Abs/ lung IgA	Yes
Yang et al. (2004)	Plasmid DNA vector	Mice	Neutralizing Abs	Yes
See et al. (2006)	Adenovirus vector	Mice	Neutralizing Abs	Yes
Bisht et al. (2004)	MVA vector	Mice	Neutralizing Abs	Yes
Chen et al. (2005)	MVA vector	Macaques	Neutralizing Abs	Yes
Weingartl et al. (2004)	MVA vector	Ferrets	No neutralizing Abs	No
Buchholz et al. (2004)	Parainfluenza virus vector	Hamsters	Neutralizing Abs	Yes
Bukreyev et al. (2004)	Parainfluenza virus vector	Macaques	Neutralizing Abs	Yes
Kapadia et al. (2005)	VSV vector	Mice	Neutralizing Abs	Yes
Vogel et al. (2007)	VSV vector	Aged mice	Neutralizing Abs	Yes
DiNapoli et al. (2007)	NDV vector	African green monkeys	Neutralizing Abs	Yes
Deming et al. (2006)	VEEV vector	Aged mice	Neutralizing Abs	Yes
		Aged mice and heterologous challenge	No neutralizing Abs	No

[a] Only those SARS-CoV vaccines containing the spike protein/gene and tested for protection against a SARS-CoV challenge are listed.
[b] MVA, modified vaccinia virus Ankara; VSV, vesicular stomatitis virus; NDV, Newcastle disease virus; VEEV, Venezuelan equine encephalitis virus.
[c] Presence of neutralizing antibodies at time of challenge.

vaccine elicited high levels of antigen-specific IgG in serum and significant levels of antigen-specific lung IgA (Hu et al., 2007). In contrast, mice immunized intramuscularly with Alum absorbed S protein did not develop detectable IgA responses. Following virus challenge of the aged mice, no virus was detected in the lungs of mice vaccinated intranasally, whereas intramuscularly immunized mice did not show significant control of virus replication compared to controls (Hu et al., 2007).

Vectored Vaccines

A DNA vaccine encoding the S glycoprotein of the SARS-CoV induces T cell, neutralizing antibody responses, and protective immunity in a mouse model (Yang et al., 2004). These authors also demonstrated that antibody responses in mice vaccinated with an expression vector encoding a form of S protein that includes its transmembrane domain elicited neutralizing antibodies. Viral replication was reduced by more than six orders of magnitude in the lungs of mice vaccinated with these S protein 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 (Kong et al., 2005). Apart from this study, several other groups have analyzed the immunogenicity of SARS DNA vaccines but none of these challenged the vaccinated animals with SARS-CoV.

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 the S protein and T cell responses against the nucleocapsid (Gao et al., 2003). More recently, See et al. (2006) demonstrated that vaccination of C57B/L6 mice with adenovirus type 5-expressing S and nucleocapsid administered intranasally, but not intramuscularly, significantly limited SARS-CoV replication in the lungs.

The highly attenuated modified vaccinia virus Ankara (MVA) has been used to express the S glycoprotein of SARS-CoV in vaccination experiments using mouse, ferret, and rhesus monkey models (Bisht et al., 2004; Chen et al., 2005; Weingartl et al., 2004). Intranasal and intramuscular administration of MVA encoding the SARS-CoV S protein led to the induction of a humoral immune response in BALB/c mice, as well as reduced viral titers in the respiratory tract (Bisht et al., 2005).

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 S protein administered via the respiratory tract induced the production of SARS-CoV neutralizing antibodies (Bukreyev et al., 2004). 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 (Buchholz et al., 2004). In the absence of S protein, 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.

Recombinant rabies virus expressing the S protein of SARS-CoV induced a neutralizing antibody response in mice (Faber et al., 2005). Similarly, an attenuated vesicular stomatitis virus vector that encodes the SARS-CoV S protein may be used to induce neutralizing antibody responses (Kapadia et al., 2005). Mice vaccinated with recombinant vesicular stomatitis virus expressing S protein developed SARS-CoV-neutralizing antibody and were able to control a challenge with SARS-CoV performed at either 1 month or 4 months after a single vaccination. In addition, by passive antibody transfer experiments these authors demonstrated that the antibody response induced by the vaccine was sufficient to control SARS-CoV infection.

The efficacy of these vectors was further demonstrated in studies using aged mice. In aged mice, vaccinated with recombinant vesicular stomatitis virus expressing S protein, antibody titers induced were sufficient to protect them against subsequent challenge with SARS-CoV (Vogel et al., 2007).

African green monkeys immunized via the respiratory tract with two doses of a recombinant Newcastle disease virus encoding the S protein developed a relatively high titer of SARS-CoV neutralizing antibodies and upon challenge demonstrated a 1000-fold reduction in pulmonary SARS-CoV titer compared with control animals (DiNapoli et al., 2007).

Finally, Venezuelan equine encephalitis virus based vaccines have been tested extensively in young and aged mice. Most importantly, different recombinant SARS-CoV bearing epidemic and zoonotic S protein variants were used to challenge the vaccinated mice. Venezuelan equine encephalitis virus replicon particles expressing the 2003 epidemic Urbani SARS-CoV strain S glycoprotein but not particles containing the nucleocapsid protein from the same strain provided complete short- and long-term protection against homologous strain challenge in young and senescent mice (Deming et al., 2006). Although the S protein encoding vaccine provided complete short-term protection against heterologous (strain GD03) challenge in young mice, only limited protection was seen in vaccinated senescent animals. Interestingly, nucleocapsid-encoding vaccines not only failed to protect from homologous or heterologous challenge but also resulted in enhanced immunopathology with eosinophilic infiltrates within the lungs of SARS-CoV-challenged mice (Deming et al., 2006).

BASIC SCIENCE AND RATIONALE OF NEW GENERATION VACCINES

A new generation of vaccines may be obtained from manipulating the full-length infectious cDNA clone of SARS-CoV. One approach would be to delete the ORFs 3a, 3b, 6, 7a, 7b, 8a, 8b, or 9b similar to other coronavirus mutants generated previously; some mouse hepatitis or feline infectious peritonitis deletion viruses replicate to the same extent as wild-type viruses in vitro but are severely attenuated in vivo making them potential vaccine candidates. However, SARS-CoV deletion mutants lacking ORFs 3a, 3b, 6, 7a, or 7b, grew similar to that of the parental wild-type virus in the mouse model (Yount et al., 2005). On the other hand, a recombinant SARS-CoV that lacks the E gene was attenuated both in vitro and in vivo (DeDiego et al., 2007). Viable recombinant virus with the E gene deleted was recovered in Vero cells with a titer around 10^6pfu/ml but titers in the respiratory tract of hamsters were 100–1000-fold reduced compared to wild-type SARS-CoV replication, suggesting that this mutant is attenuated. Multiplication of these viruses in packaging cell lines would provide the missing protein in trans and would make a promising SARS-CoV vaccine candidate that has the E gene deleted.

Live attenuated virus vaccines may revert to wild-type and recombine with other circulating human or

zoonotic coronaviruses. In order to prevent this, it has been proposed to delete an essential gene, located in a position distant from gene E, and the relocation of the deleted gene to the position previously occupied by gene E (Enjuanes et al., 2008). A potential recombination leading to the rescue of gene E would lead to the loss of the essential gene. Alternatively, the transcriptional regulatory sequences (TRS) of a vaccine virus could be genetically manipulated to a sequence incompatible with the TRS of any known circulating coronavirus as described by Yount et al. (2006). This virus could be further modified by building attenuating mutations on the genetic backbone of the recombination resistant TRS rewired virus either for use as a safe high titer seed stock for making killed vaccines or as a live virus vaccine. One such attenuating mutation could be targeted to the nonstructural protein 1. Recombinant Mouse Hepatitis Virus (MHV) encoding a deletion in the nsp1-coding sequence grew normally in tissue culture but was severely attenuated in vivo (Züst et al., 2007). Low doses of nsp1 mutant MHV elicited potent cytotoxic T cell responses and protected mice against homologous and heterologous virus challenge. This attenuation strategy provides a new paradigm for the development of highly efficient coronavirus vaccines.

PRECLINICAL DEVELOPMENT, INCLUDING RELEVANT ANIMAL MODELS

Although several types of vectored vaccines have been developed, several companies favored the classical approach using inactivated whole virus to develop a vaccine to be used for preclinical testing (see Chapter 11). Methods in place for the production of available vaccines could be easily used using well-established technologies. In the preclinical development stage, it is preferable that different animal species are used to evaluate the safety and efficacy of candidate vaccines.

Overall, a wide range of animal species, including rodents (mice and hamsters), carnivores (ferrets and cats), and nonhuman primates (cynomolgus and rhesus macaques, common marmosets, and African green monkeys) can be experimentally infected with SARS-CoV (Subbarao et al., 2004; Roberts et al., 2005b; Martina et al., 2003; Kuiken et al., 2003; McAuliffe et al., 2004; Haagmans and Osterhaus, 2006). 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 (Roberts et al., 2005a). In contrast, inoculation of SARS-CoV in the respiratory tract of cynomolgus macaques causes infection of bronchial epithelial cells and type-1 pneumocytes 1–4 days postinfection, followed by extensive type-2 pneumocyte hyperplasia in the lungs at 4–6 days postinfection (Kuiken et al., 2003; Haagmans and Osterhaus, 2006). The lesions, consisting of multiple foci of acute diffuse alveolar damage and characterized by flooding of alveoli with protein-rich edema fluid mixed with variable numbers of neutrophils, are quite similar to those observed in humans in the acute stages of SARS.

Remarkably, vaccine candidates tested in ferrets showed reduced efficacy. Vaccination with MVA encoding the S protein induced only moderate antibody responses and consequently did not protect against intranasal SARS-CoV infection and even resulted in an inflammatory response in the livers of the vaccinated ferrets (Weingartl et al., 2004). Whether these aberrant responses resulted from immunopathological mechanisms, like antibody-dependent enhancement of infection, or represented recall responses to viral antigen in the liver is not clear at the moment but deserves further investigation. In addition, limited protection from SARS-CoV challenge was observed in ferrets vaccinated with inactivated whole virus (Darnell et al., 2007). Two out of four ferrets showed little or no neutralizing antibody even after the second immunization and none of the vaccinated ferrets were able to reduce virus excretion at day 2 after challenge but subsequently cleared the virus more rapidly compared to control animals. Adenovirus-based vaccines tested in ferrets seemed more powerful as they protected the lower respiratory tract efficiently but had less effect on virus excretion in the upper respiratory tract (Kobinger et al., 2007).

CLINICAL TRIALS

Five years after the first SARS outbreak a range of candidate vaccines have been developed. Early in 2006 some companies in China and the US initiated phase 1 trials. The first clinical trial has been initiated by a Chinese company, Sinovac Biotech of Beijing in collaboration with the Chinese academy of Medical Sciences using an inactivated whole virus vaccine. To evaluate the safety and immunogenicity of this vaccine, 36 subjects received two doses of vaccine or placebo control. On day 42, all individuals showed seroconversion and peak titers of neutralizing antibodies were reached 2 weeks after the second vaccination followed

by a significant decline 4 weeks later (Lin et al., 2007). Several other candidate SARS vaccines are at various stages of preclinical and clinical development.

POSTEXPOSURE IMMUNOPROPHYLAXIS

In SARS patients who 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 (Zhang et al., 2006). 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 (Subbarao et al., 2004). 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-CoV in the lungs of infected ferrets by 1000-fold, completely prevented the development of SARS-CoV-induced macroscopic lung pathology, and abolished shedding of virus in pharyngeal secretions (Ter Meulen et al., 2004). In subsequent studies, several other monoclonal antibodies were evaluated for their efficacy in mouse and hamster models (Sui et al., 2005; Traggiai et al., 2004).

PROSPECTS FOR THE FUTURE

The importance of assessing immunogenicity of candidate SARS-CoV vaccines using virus neutralization assays is well acknowledged, but the variety of these 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 (Giroglou et al., 2004).

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 (Weiss and Scott, 1981). 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 S protein into cells, but not replication (Yang et al., 2005). To date, 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.

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 cause major outbreaks but vaccines, antivirals, or passive immunization would be relevant in the context of protecting high-risk individuals such as laboratory and health-care workers.

KEY ISSUES

- Five years after the first SARS outbreak, several candidate SARS-CoV vaccines are at various stages of preclinical and clinical development.
- Based on the observation that SARS-CoV infection is efficiently controlled upon passive transfer of antibodies directed against the S protein of SARS-CoV, vaccines encoding this protein have been developed.
- Animals immunized with diverse vaccines containing the S protein or S protein gene developed SARS-CoV-neutralizing antibodies and were protected against SARS-CoV challenge.
- Future challenges for the development of SARS-CoV vaccines are linked to the potential re-emergence of this virus. Vaccines able to induce highly cross-reactive antibodies which efficiently protect the gastrointestinal and respiratory tract are needed. Given the fact that in the previous outbreak mainly elderly humans succumbed to the infection, special attention should be given to protect specifically these individuals.

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