# Chapter 13
# SARS Coronavirus Pathogenesis and Therapeutic Treatment Design

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**Abstract** Emerging pathogens are either new or newly recognized or those that are increasing in incidence and spread. Since the identity of emerging pathogens from animal reservoirs is difficult to predict, the development for pathogen-specific therapeutics and vaccines is problematic. The highly pathogenic SARS coronavirus (SARS-CoV) emerged from zoonotic pools in 2002 to cause a global epidemic of severe acute respiratory syndrome (SARS). Many patients with SARS-CoV experienced an exacerbated form of disease called acute respiratory distress syndrome (ARDS) requiring mechanical ventilation and supplemental oxygen and half of these patients died. Similar to other viral pathogens like influenza and West Nile Virus, the severity of SARS-CoV disease increased with age. Unfortunately, successful vaccination in the most vulnerable populations is a difficult task because of immunological deficiencies associated with aging (immune senescence). Due to the rapidity of virus emergence, technologies like synthetic biology can be harnessed to facilitate rapid recombinant virus construction for studying the novel virus biology, pathogenesis and the evaluation of therapeutic interventions. Since predicting the antigenic identity of future emergence is difficult, candidate vaccines and therapeutics should have a maximal breadth of cross-protection, and panels of antigenically divergent synthetically reconstructed viruses can be used as tools for this evaluation. We discuss how synthetic reconstruction of many animal and human SARS-CoV has provided a model to study the molecular mechanisms governing emergence and pathogenesis of viral diseases. In addition, we review the evolution, epidemiology, and pathogenesis of epidemic and zoonotic SARS-CoV with focus on the development of broadly reactive therapeutics and vaccines that protect aged populations from the zoonotic pool.

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## 13.1 Introduction

Many diseases of great human historical significance in the ancient and recent past like plague, smallpox, HIV, and influenza A emerged from wild or domestic animal populations to cause devastating human disease (Achtman et al. 1999; Li et al. 2007; Nguyen et al. 2005; Parrish and Kawaoka 2005; Tumpey et al. 2005; Wolfe et al. 2007; Woo et al. 2006). In 2002, a novel coronavirus (SARS-CoV) emerged suddenly as the causative agent of severe acute respiratory syndrome (SARS) and spread worldwide causing about 8,000 cases and >700 deaths (Christian et al. 2004; Ksiazek et al. 2003; Rota et al. 2003). Viruses similar to the epidemic strain were isolated from civets for sale within wet markets in China during the epidemic in 2003 and the reemergence of 2004 (Chinese 2004; Guan et al. 2003). Genome sequences of viruses isolated from bats, civets and humans suggest that viruses circulating in bats crossed the species barrier to infect civets who then served as an amplification host for yet another host-range shift to generate human tropic virus (Chinese 2004; Guan et al. 2003; Lau et al. 2005; Li et al. 2005b). Since viruses similar to the epidemic strain of SARS-CoV are currently circulating in zoonotic pools, the future emergence of a SARS-CoV-like virus may occur, as has occurred with Ebola, influenza H5N1, Marburg, and chikungunya virus (Gonzalez et al. 2007; Kaur et al. 2008; Leroy et al. 2005; Towner et al. 2007; Woo et al. 2006). Therefore, it is imperative that we understand the pathogenic mechanisms of coronavirus lung diseases and that current vaccination and passive sero-therapies be effective in protecting humans from infection by zoonotic SARS-CoV. Though a considerable amount of work has enhanced our knowledge of SARS-CoV pathogenesis and therapeutic treatment design, many questions remain unanswered. What host factors have contributed to the protection or prevention of severe disease? Will SARS-CoV therapeutics be effective against future emergence? How do we rationally design an antiviral therapy against future emergence of unknown antigenic identity? Will SARS-CoV therapeutics protect the most vulnerable human populations? The current research aimed at answering these questions is the focus of this review.

## 13.2 Human SARS-CoV Pathogenesis

### *13.2.1 The Clinical Course of Human SARS-CoV Infection*

SARS-CoV is thought to have emerged suddenly from zoonotic pools of virus. Molecular epidemiology suggests that the epidemic strain evolved from bat-associated SARS-CoV-like viruses by way of a intermediate civet host (Fig. 13.1). Without SARS-CoV evolution promoting efficient infection of human cells and person-to-person transmission, the emergent SARS-CoV epidemic would not have occurred. Thus, zoonotic SARS-CoV adaptation was a necessary initial step in

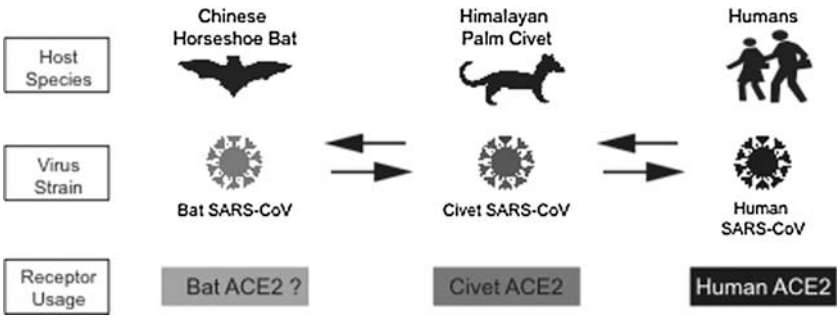



**Fig. 13.1** Schematic of SARS-CoV emergence from zoonotic pools. Viruses similar to the epidemic strain of SARS-CoV, SARS Urbani, were found within Chinese horseshoe bats, which are believed to be the animal reservoir for SARS-CoV like viruses. Within live animal markets in China, it is postulated that interaction between bat and Himalayan palm civets helped the virus to jump species to infect civets. Viruses more closely related to SARS Urbani were found in civets in markets during the epidemic. Over time, civet viruses were transmitted to humans, ultimately resulting in a virus that could be transmitted from person to person. During the early middle and late phases of the epidemic, SARS-CoV evolved further until the virus stabilized as SARS Urbani

SARS-CoV human pathogenesis. Sequence analysis of zoonotic, early, middle and late-stage epidemic strains, coupled with in vitro evolution experimentation, has demonstrated that zoonotic isolates can rapidly adapt to efficient growth in human airway cells by multiple genetic pathways (Chinese 2004; Guan et al. 2003; Li et al. 2005a, 2005b; Sheahan et al. 2008a, 2008b). The plasticity of the SARS-CoV spike (S) glycoprotein and receptor interaction is a particularly troubling harbinger for the ease and potential of future cross-species transmission. SARS-CoV is thought to be transmitted by direct patient contact, airborne droplet nuclei, contact with fomites or urine/fecal contact with mucous membranes (Peiris et al. 2003a, 2003b). After a brief incubation period of approximately 6 days, the patient enters the acute phase of infection characterized by fever (>100°F), chills, malaise, and myalgia (Booth et al. 2003; Liang et al. 2004; Peiris et al. 2003a). During the acute phase of the infection patients develop a nonproductive cough/shortness of breath (dyspnea), and bilateral pulmonary infiltrates are seen by chest radiography. Pulmonary lesions visible by radiography continue to worsen until 7 days after the onset of symptoms (AOS), after which most patients begin to improve. Approximately 30% of patients show clinical improvement after the first week of illness while the remaining 70% present with recurring fever and shortness of breath (Peiris et al. 2003a, 2003b). A case study of health care workers in Toronto ($n = 14$, age mean = 42 years, age range 27–63 years) provides a typical example of the course of SARS-CoV convalescence, where a week after hospital discharge, all patients complained of dyspnea, weakness, and lethargy and all suffered from significant weight loss (anorexia) from SARS-CoV disease (Avendano et al. 2003). Three weeks after discharge, the Toronto healthcare worker cohort were no longer weak and continued to gain weight but still suffered from dyspnea (14/14 patients) and a

few still presented with an abnormal chest X-ray (5/14 patients) (Avendano et al. 2003). A case study in Hong Kong provides a detailed example of a nonconvalescent cohort where lung damage continues to progress in a minority (20–30%) of patients where "diffuse ground glass" changes are seen in the chest X-ray typical of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) (Peiris et al. 2003a). ALI can progress to ARDS, which is characterized primarily by an acute onset, bilateral infiltrates on chest radiograph, hypoxemia, fever, and leukopenia (Bauer et al. 2006a).

ALI and ARDS are inflammatory lung diseases characterized by diffuse alveolar infiltration, hypoxia, respiratory failure, and death due to the failure of multiple organs. The most severe form of ALI, ARDS, is fatal in almost 50% of patients and affects ~1,000,000 people/year worldwide, ranking it among the most difficult challenges in critical-care medicine (Kuba et al. 2006a). ARDS is characterized by diffuse alveolar damage (DAD), which includes a protein-rich edema, an exudative phase with hyaline membranes, and inflammation leading to surfactant dysfunction and severe hypoxia. Neutrophils dominate in bronchoalveolar lavage (BAL) fluid, and cytokines TGF-β1, TNFα, IL-1β, IL-6, IL8 are often elevated (Bauer et al. 2006b; Dahlem et al. 2007; Guidot et al. 2006). The exudative phase lasts about a week, and a progressive proliferative phase can progress and organize, resulting in fibrosis (fibrotic phase). Approximately 20% of SARS patients required intensive care unit (ICU) treatment due to ARDS symptoms and a majority of those admitted to the ICU require mechanical ventilation and oxygen support (Booth et al. 2003; Peiris et al. 2003a). Though overall mortality rates of the global SARS-CoV epidemic approached 8%, the mortality rates for patients over the age of 65 ranged from approximately 25 to 55% as a result of comorbidities and immune senescence (Booth et al. 2003; Leung et al. 2004; Liang et al. 2004; Peiris et al. 2003a).

In the lung, the primary target cells of SARS-CoV infection of humans remains controversial. Several thorough pathological studies of post-mortem tissues from SARS-CoV infection have identified the ciliated epithelial cells, and type I and II pneumocytes within the lung as the primary target of virus infection, though virus antigen has also been found in macrophages (Mø), dendritic cells (DCs), T cells, B cells, NK cells, and putative lung stem/progenitor CD34 + Oct-4+ cells (Chen et al. 2007; Gu et al. 2005; Hwang et al. 2005; Nicholls et al. 2006; Tse et al. 2004; Ye et al. 2007). Unfortunately, the in-situ hybridization utilized in these pathological studies was unable to differentiate between active viral infection and uptake of virus by passive cellular means like phagocytosis. Several in vitro studies have demonstrated that human Mø and DCs were unable to support active virus replication and instead instigated cell activation leading to upregulation of MHC II and secretion of inflammatory cytokines (Cheung et al. 2005; Law et al. 2005; Tseng et al. 2005). In support of gross lung pathology seen by X-ray, microscopic evaluation of SARS-CoV lung pathology has repeatedly been described in various cohorts as showing various phases of exudative and proliferative acute lung injury (ALI) (Gu et al. 2005; Hwang et al. 2005; Nicholls et al. 2006; Tse et al. 2004). Typical SARS-CoV lung pathology is characterized by inflammatory cell infiltration, pulmonary edema, hyaline membrane formation, mild to moderate fibrosis, alveolar epithelial

hyperplasia, and alveolar/epithelial cell desquamination (i.e., sloughing) (Gu et al. 2005; Hwang et al. 2005; Tse et al. 2004). In two postdischarge cohorts, the percentages of patients with continued symptoms and pulmonary fibrosis ranged from 21 to 62% and the development of fibrosis was associated with increasing age and admission to intensive care (Antonio et al. 2003; Wang et al. 2008). Pulmonary fibrosis (PF) is a devastating disease with an almost universally fatal outcome characterized by inflammation of the alveoli, damage to lung tissues, and progressive interstitial fibrosis (hardening of tissues). There are five million people worldwide that are affected by PF, including some 200,000 cases culminating in 40,000 deaths/year in the US (http://www.pulmonaryfibrosis.org/home.htm). Models of virus-induced PF are essential for understanding and managing these devastating end-stage clinical diseases. The ultimate clinical course of ARDS is often determined by the ability of the injured lung to repopulate the alveolar epithelium with functional cells. Death may occur when fibrosis predominates during the healing response, worsening lung compliance and oxygenation.

### *13.2.2 The Human Adaptive Immune Response to SARS-CoV*

Chest X-ray, serology and virological data suggest direct involvement of the adaptive immune response in viral clearance. A detailed virological/immunological longitudinal analysis was performed on a cohort of SARS patients in Hong Kong (Peiris et al. 2003a). Five days AOS, nasopharyngeal aspirates contained between 3 and 7 $\log_{10}$ genomes/ml of SARS-CoV and by day 10 the range tightened to 5–7 $\log_{10}$ genomes/ml. In most patients SARS-CoV-specific IgG seroconversion begins near day 10 AOS (mean = 20 days AOS) after which virus titers begin to fall. Interestingly, 40% of the cohort ($n = 75$) did not seroconvert until 24 days AOS but 93% had seroconverted by day 29 AOS. Even after seroconversion, viral genomes were detected in nasopharyngeal aspirate (47%), stool (67%) and urine (21%) as far as 21 days AOS. Of note, many patients in the Hong Kong cohort were treated with corticosteroids and these drugs may have delayed the onset of seroconversion. In an animal model of coronavirus lung infection (PRCV) with similar pathologies to SARS-CoV, the administration of corticosteroids alleviated signs of PRCV pneumonia early (2 dpi) though exacerbated later stages of disease (4, 10, 21 dpi) probably due to the lack of a cell-mediated response creating an environment for extended virus lung replication (Jung et al. 2007). Together, these data suggest that the administration of corticosteroids during SARS-CoV acute infection may exacerbate disease due to dampening of the cell-mediated immune response. In another Hong Kong SARS cohort, seroconversion was detected as early as 4 days AOS with a median seroconversion occurring at 15 days AOS (Lee et al. 2006). Lastly, Cameron et al. correlated elevated levels of anti-SARS-CoV S-specific antibody in patient sera with less-severe disease (Cameron et al. 2007). Nevertheless, these data suggest that as the adaptive immune response mounts, viral load is depressed paving the way for convalescence. Unfortunately, all current animal models fail to recapitulate the adaptive immune response to SARS-CoV.

### 13.2.3 The Human Innate Immune Response to SARS-CoV

Important insights into the mechanisms of the innate inflammatory response of SARS-CoV infection has been gleaned from several clinical, in vitro and in vivo animal model studies, yet a clear and concise model of this innate response and its relation to viral pathogenesis remains to be elucidated. Inconsistencies in experimental protocol, clinical treatment, cell type infected in vitro, and possible species-specific effects within various animal models have created a confusing and complicated body of data making the generation of a comprehensive model of SARS-CoV pathogenesis difficult. Nevertheless, concordant data between experimental systems has provided the coronavirus field with a great body of useful information and those data are summarized below.

A thorough evaluation of inflammatory gene expression in SARS-CoV patient peripheral blood mononuclear cell (PBMC) was performed by both Cameron et al. and Regunathan et al. but the investigators arrived at disparate conclusions. Cameron et al. concluded that a robust type I INF response was observed early in the progression of disease and was predicted to be essential for viral clearance and convalescence (Cameron et al. 2007). In addition to a strong INF response, genes typically induced by INF like CCL2 (MCP-1) and CXCL10 (IP-10) were also upregulated in patients early during SARS-CoV disease (Cameron et al. 2007). Interestingly, Cameron and collaborators present data that suggest that the cytokine storm that serves to protect some SARS-CoV patients can progress in an unchecked manner and contribute to the development of an inadequate adaptive immune response and more severe disease (Cameron et al. 2007). Reghunathan and collaborators also sampled SARS patient PBMCs for microarray analysis but did not find type I INF induced in their samples and instead found upregulation of several other tissue inflammation/remodeling, homeostasis, and cell-cycle genes (Reghunathan et al. 2005). Compounding the fact that Reghunathan did not discuss virological data and failed to report the stage of SARS-CoV disease at which these samples were extracted (acute, convalescent, etc.), the sample size ($n = 10$) was small in comparison to the Cameron et al. cohort ($n = 50$) (Cameron et al. 2007; Reghunathan et al. 2005). Of note, many cohorts of SARS patients, including the Cameron cohort discussed above, were treated with immunosuppressive corticosteroids making the resulting immunological and virological data more difficult to interpret and evaluate (Stockman et al. 2006).

### 13.2.4 Animal Models of SARS-CoV Pathogenesis

Since human clinical SARS data is complicated by host genetic variation, disease-exacerbating comorbidities, age variation, and variable drug-treatment regimens, animal models provide a more homogeneous and controlled environment within which to ask questions related to the involvement of the host immune response to SARS-CoV infection. In nonhuman primate (NHP, cynomolgus macaque) infection

with SARS-CoV, Haagmans et al. demonstrated the prophylactic administration of pegylated INFα controlled virus replication and lessened disease pathology (Haagmans et al. 2004). Concordantly, transcriptional profiles of NHP-infected lung tissue suggest that INFα, β, λ, and γ and also IP-10, MCP-1, IL-6, and IL-8 genes were all upregulated in SARS-CoV-infected NHPs (de Lang et al. 2007). Since NHPs do not succumb to infection, it is proposed that the inflammatory response to virus infection described above aids in the control and clearance of this acute infection.

The development of mouse models that recapitulate components of human disease have been invaluable in viral pathogenesis research. Young BALB/c and C57BL/6 strains of mice support SARS Urbani replication in the lung and infection causes lung pathology similar to that seen in human cases, but these models lack both morbidity and mortality and the more severe lung pathologies noted in human patients (Roberts and Subbarao 2006; Rockx et al. 2007). Since replication models provide little utility regarding disease pathogenesis, several models of severe SARS-CoV disease were developed in 2007 (Table 13.1). Subbarao et al. created a mouse-adapted SARS-CoV (MA15) through repeated passage of SARS Urbani in BALB/c mice (Roberts et al. 2007). Our laboratory created a molecular clone of MA15 (rMA15 ) through the introduction of the six mouse-adapting amino acid changes into our infections clone for SARS Urbani (icSARS) (Roberts et al. 2007) (Fig. 13.2). When administered intranasally to young adult BALB/c mice (6–10 weeks), rMA15 causes significant weight loss (~20% of starting weight) resulting from a severe acute infection of the lung, resulting in almost 100% mortality by 4 dpi (Roberts et al. 2007). rMA15 mortality ensues more rapidly with increasing age in BALB/c mice, where year-old mice succumb to infection beginning on 3 dpi (unpublished observation, Sheahan and Baric). Similarly, Rockx et al. developed an age-related mouse model of severe SARS-CoV disease where infection of old BALB/c mice with SARS-CoV bearing the civet HCSZ61/03 or early human GZ02 S glycoprotein genes caused uniform mortality by 4 dpi and lung pathologies quite similar to those seen in human cases (Rockx et al. 2007). Due to the acute and severe nature of the infections within these lethal models (100% mortality by 4 dpi), many aspects of natural SARS-CoV infection such as development of the adaptive immune response, death after virus clearance due to immunopathology, and the development of pulmonary fibrosis cannot be assessed. Since a majority of the human SARS-CoV cases resulted in patient morbidity and survival, models with morbidity but without mortality should be developed in order to study the mechanisms of host protection reflecting the prevailing course of SARS-CoV disease.

Though current animal models are not able to assess the importance of adaptive immunity in SARS-CoV pathogenesis, multiple studies have implicated the importance of the innate response in viral clearance. Glass et al. demonstrated that SARS-CoV was cleared with similar kinetics in WT C57BL/6 mice or strains that lacked that T, B and NK cells, suggesting that the innate immune response alone is sufficient for viral clearance (Glass et al. 2004). Like gene expression data from human samples, MCP-1, MIP1-α, and IP-10 are all upregulated in the lung during SARS-CoV infection of C57BL/6 mice, suggesting a role for these chemokines in

**Table 13.1** Models of lethal disease for studying SARS-CoV pathogenesis and passive immunization/vaccine efficacy

| Virus name | Description | Total AA Δs from Urbani | Spike AA Δs from Urbani | Pathogenic phenotype | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Young BALB/c | Aged BALB/c | Young C57BL/6 | Aged C57BL/6 |
| icSARS | Molecular clone of the epidemic strain SARS Urbani | 0 | 0 | Weight loss | + | ++ | No | No |
| | | | | Replication in lung | ++ | +++ | ++ | ++ |
| | | | | Mortality | No | +/− | No | No |
| icGZ02 | icSARS bearing an early epidemic phase virus spike | 6 | 6 | Weight loss | + | +++ | N/A | N/A |
| | | | | Virus replication in lung | ++ | +++ | N/A | N/A |
| | | | | Mortality | No | Yes | N/A | N/A |
| icHC/SZ/61/03 | icSARS bearing a zoonotic civet SARS-CoV spike | 21 | 21 | Weight loss | + | +++ | N/A | N/A |
| | | | | Virus replication in lung | ++ | ++ | N/A | N/A |
| | | | | Mortality | No | Yes | N/A | N/A |
| rMA15 | Molecular clone of the mouse adapted SARS-CoV | 6 | 1 | Weight loss | +++ | +++ | ++ | +++ |
| | | | | Virus replication in lung | +++ | +++ | ++ | +++ |
| | | | | Mortality | Yes | Yes | No | Yes |
| rMA15 GD03-S | rMA15 bearing the GD03 spike | 24 | 19 | Weight loss | ++ | +++ | N/A | N/A |
| | | | | Virus replication in lung | ++ | +++ | N/A | N/A |
| | | | | Mortality | No | Yes | N/A | N/A |

N/A: Not accessed; AA Δs: amino acid changes

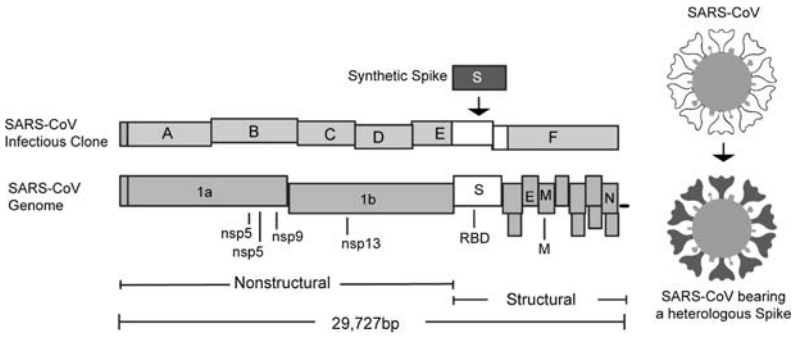



**Fig. 13.2** SARS-CoV reverse genetics, mouse adapting mutations and the construction of SARS-CoV bearing heterologous spike proteins. The SARS-CoV infectious cDNA clone for the epidemic strain, SARS Urbani, developed in the Baric laboratory divides the 29,727 bp virus genome over six cDNA plasmid clones. To construct a SARS-CoV bearing a heterologous spike (S) protein, synthetic biology can be harnessed and the heterologous S gene can be inserted into the infectious clone using standard molecular biology techniques. When the recombinant virus is constructed, only the S gene is heterologous, resulting in a SARS-CoV bearing a heterologous S protein. The locations of the mouse-adapting mutations reported by Roberts et al. (2006) are marked within the schematic of the SARS-CoV genome (nsp5, nsp5, nsp9, nsp13, Spike RBD, and M)

protection (Glass et al. 2004). In support of Glass et al., Hogan et al. demonstrated that STAT1, a key modulator of INF α/β, λ, and γ signaling, was required for the resolution of SARS infection, once again implicating the importance of the innate response in the clearance of SARS-CoV (Hogan et al. 2004). The induction of INF in mice seems to be dependent on mouse strain ,where BALB/c mice induce type I INF following SARS-CoV infection as measured by microarray (Rockx et al. 2009) and ELISA while the induction of INF is undetectable in C57BL/6 mice (Sheahan et al. 2008) (Roberts et al. 2005a). We have recently developed a C57BL/6 mouse model of acute SARS-CoV pathogenesis with significant morbidity (12–15% loss in body weight) and complete recovery due to the activation of the innate response and the recruitment of inflammatory leukocytes to the lung (see Sect. 13.2.5) (Sheahan et al. 2008). These immunologic and pathologic discrepancies between mouse strains are unfortunate caveats of animal models of viral disease. Moreover, no animal model of SARS-CoV pathogenesis to date has fully recapitulated both the innate and adaptive immunopathological aspects SARS-CoV disease. Nevertheless, mouse models of SARS-CoV pathogenesis faithfully recapitulate many aspects of acute human disease like virus replication, the induction of inflammatory cytokines, migration of immune cells into pulmonary tissues, virus- and immune-cell-mediated lung pathology, and weight loss. Also, unlike human and NHP in vivo models, transgenic "knock out" mice provide the opportunity to evaluate the role of single genes in viral pathogenesis. Recently, a novel panel of genetically dissimilar recombinant inbred mice has been developed called "the collaborative cross," which will allow for the elucidation of genetic pathways involved in multigenic complex traits (Churchill et al. 2004). The genetic diversity within the collaborative cross is similar to that present in the human population and, using

statistical and genomic analysis, genes responsible for observed phenotypes are elucidated. Rather than evaluate the role of a single gene in SARS-CoV pathogenesis in "knock out" mice, the use of the collaborative cross for SARS-CoV pathogenesis may uncover roles for multiple host genes involved in the progression or prevention of disease.

### *13.2.5 In Vitro Models of SARS-CoV Pahtogenesis*

Perhaps the most simplified models within which to study SARS-CoV pathogenesis are in vitro models. Though in vitro models are less complicated than in vivo models, the resultant data and relevance to SARS-CoV pathogenesis is hotly debated. There is a seeming incongruity between in vivo human, primate and mouse data where the induction of INF is observed while the in vitro infection of various primary or immortalized interferon-competent cell types fail to induce or produce INF. These in vitro experiments are further complicated by the notion that several viral genes have been implicated as active INF antagonists (Frieman et al. 2007; Kopecky-Bromberg et al. 2007; Zust et al. 2007). The in vitro data regarding SARS-CoV innate immune activation is reviewed below.

The infection of interferon-competent primary human airway epithelial cells (HAE) with SARS-CoV does not result in the induction or secretion of INF but does result in the secretion of inflammatory chemokines IL-6, MCP-1 and IP-10 (Sims unpublished data) (Frieman et al. 2007). Pathological evaluation of lung tissue from lethal SARS-CoV and in vitro data suggests that airway epithelial cells are the primary target for SARS-CoV infection yet do not induce INF in vitro (Sims et al. 2005; Tse et al. 2004; Ye et al. 2007). In NHP studies by Haagmans and colleagues, it was demonstrated that epithelial cells adjacent to infected epithelial cells stained positive for INFβ, suggesting a possible bystander activation effect. It may be that viral INF antagonists suppress the antiviral-sensing network in the infected cell but eventually neighboring cells are activated by circulating interferons released from nonpermissive cells or through the sensing of viral proteins or genomic RNA released into the extracellular milieu as a result of viral-induced cell lysis.

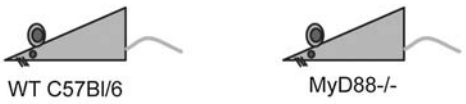
Several studies focusing on infection of professional antigen-presenting cells like dendritic cells and macrophages have also produced controversial data. Most studies utilize human PBMC-derived macrophages or DCs where CD14+ cells are isolated and differentiated in the presence of cytokines (Mø = GM-CSF, DC = IL-4, GM-CSF) in vitro, after which the cell populations resemble "macrophages" and "dendritic cells" by cell surface staining profiles (Cheung et al. 2005; Frieman et al. 2007; Law et al. 2005). When SARS-CoV is added to these Mø and DC populations, a productive infection does not ensue; INF is not induced but several other inflammatory cytokines are induced and secreted (MIP1-α, IP-10, MCP-1) (Cheung et al. 2005; Frieman et al. 2007; Law et al. 2005). A possible explanation for the disparity between the in vitro and in vivo data regarding INF induction in SARS-CoV infection is presented by Cervantes-Barragan and colleagues where

they show key differences in conventional (cDC) and plasmacytoid dendritic cell (pDC) populations in response to SARS-CoV infection (Cervantes-Barragan et al. 2007). pDCs differ from cDCs in their surface characteristics (cDC = CD11c+, B220−, pDC = B220+, CD11c-low, PDCA-1+) and function where pDCs are the major source of INFα in both humans and mice (Asselin-Paturel et al. 2001; Cella et al. 1999; Cervantes-Barragan et al. 2007; Siegal et al. 1999). Unlike most investigators who artificially differentiate Mø and DC from CD14+ precursor cells isolated from PBMCs, Cervantes-Barragan and colleagues isolated cDC and pDC populations directly from human blood which were subsequently incubated with SARS-CoV and demonstrated that, unlike cDC, pDC induced INFβ transcription and produced large amounts of INFα protein in the cell media (Cervantes-Barragan et al. 2007). In the assessment of both pDC and cDC populations side by side, Cervantes-Barragan provide a possible explanation as to why previous studies of SARS-CoV "infection" of dendritic cells failed to induce INF, especially since the differentiation protocol used by Law, Tseng, and Cheung results in the differentiation of a more cDC-like cell (Cervantes-Barragan et al. 2007; Cheung et al. 2005; Law et al. 2005; Tseng et al. 2005). Cervantes-Barragan also utilized in vitro differentiated mouse cDC and pDC that were used in mouse hepatitis virus (MHV) experiments. Interestingly, the cytokine used by Cervantes-Barragan to generate cDC (GM-CSF only) from CD14+ cells was used by other investigators to create Mø, though the generation of the pDC using Flt3-L was not employed by either Law, Tseng or Cheung (Cervantes-Barragan et al. 2007; Cheung et al. 2005; Law et al. 2005; Tseng et al. 2005). Though the work of Cervantes-Barragan helps clarify some of the discrepancies seen in studies of the innate immune response to SARS-CoV, the body of work is deficient in demonstrating the generation of infectious virus through infection of cDCs or in suggesting a mechanism of SARS-CoV binding and entry, since ACE2 expression in cDCs was not addressed (Cervantes-Barragan et al. 2007; Cheung et al. 2005; Law et al. 2005; Tseng et al. 2005). Of note, studies have demonstrated that the lectins, DC-SIGN/L-SIGN, are coreceptors for SARS-CoV docking and entry and these receptors are often found on DCs and other APCs (Jeffers et al. 2004). Nevertheless, it will be interesting to see whether future mouse/NHP studies definitively demonstrate a role for pDCs in SARS-CoV pathogenesis.

### *13.2.6 SARS-CoV and MyD88*

Toll-like receptors (TLRs) and Nod-like receptors (NLRs) are examples of host cell proteins that recognize pathogen-associated molecular patterns (PAMPs) (O'Neill and Bowie 2007; Uehara et al. 2007). MyD88 is an important adaptor protein required for the perpetuation of almost all TLR proinflammatory signals as well as interleukin-1 and -18 receptor (IL-1R1, IL-18R1) signaling events (O'Neill and Bowie 2007). Recent data has implicated MyD88 in both the progression and prevention of viral disease. Infection of MyD88-deficient mice with respiratory

syncytial virus (RSV) or vesicular stomatitis virus (VSV) results in an exacerbation of disease, while infection with reovirus results in a similar clinical course to that seen in WT mice (Johansson et al. 2007; Phipps et al. 2007; Rudd et al. 2007; Zhou et al. 2007b). We have recently developed a mouse model for SARS-CoV pathogenesis that recapitulates aspects of the acute human infection where wild-type C57BL/6 mice infected with $10^5$ pfu of rMA15 (recombinant mouse adapted SARS-CoV) experience a significant but transient weight loss (12–15% by 3–4 dpi), high titer virus replication ($>10^8$pfu/g 1 and 2 dpi), inflammation in the lung with the induction of proinflammatory chemokines and cytokines with a marked recruitment of inflammatory monocytes to the infected lung, and virus clearance and recovery from disease by 7 dpi (Fig. 13.3) (Sheahan et al. 2008). Interestingly, infection of age- and sex-matched MyD88-deficient mice results in a failure to control virus replication with significantly higher lung titers over time, a delay in the induction of inflammatory gene transcription, a delay in inflammatory leukocyte recruitment, and 90% mortality by 6 dpi (Sheahan et al. 2008). The receptor providing the protective signal through MyD88 remains to be elucidated though we have ruled out both IL-1R1 and IL-18R1. These data suggest that the MyD88-dependent induction of innate proinflammatory chemokines and cytokines and the subsequent recruitment of inflammatory leukocytes are required for protection from




| | WT C57Bl/6 | MyD88-/- |
|---|---|---|
| Peak Titer | 2dpi $10^9$ pfu/g | 2dpi $10^9$ pfu/g |
| Virus clearance | by 7dpi | Persistant infection $> 10^7$ pfu/g until death |
| Weight Loss | 12-15% by 3-4dpi | 12-15% by 3-4dpi |
| Pulmonary proinflammatory gene transcription | Massive upregulation of IL-1, IL-6, TNF, CCL2, CCL3, and CCL5 by 2dpi | Minimal upregulation of IL-1, IL-6, TNF, CCL2, CCL3, and CCL5 by 4dpi |
| Recruitment of inflammtory leukocytes | Moncytes and Neutrophils by 2dpi | Moncytes and Neutrophils by 4dpi |
| Lung Pathology | Mild bronchiolitis, inflammatory cell recruitment (2dpi) | Severe denuding bronchioltis, delayed inflammatory cell recruitment (4dpi) |
| Mortality | No | Yes |

**Fig. 13.3** Phenotypic differences between mouse-adapted SARS-CoV (rMA15)-infected WT C57BL/6 or mice deficient in the gene *MyD88*. MyD88 is an important adapter protein for almost all toll-like receptors, IL-1R1, and IL-18R1 proinflammatory signaling events. Mice deficient in MyD88 are far more susceptible to rMA15 infection

SARS-CoV-induced mortality, and future studies may elucidate the precise interaction between the virus and host responsible for the MyD88-dependent protective signal. Furthermore, future genetic and epidemiological studies of SARS-CoV-infected persons may reveal a role for MyD88- and MyD88-related gene polymorphisms in SARS-CoV disease.

### *13.2.7 SARS-CoV and the Renin–Angiotensin System*

The cellular receptor for SARS-CoV infection, angiotensin I converting enzyme 2 (ACE2), serves as a prime example of a cellular protein strictly required for the virus to gain entry into the host cell while also serving an important function in host physiology and perhaps viral pathogenesis and disease. Within a year of discovering SARS-CoV, ACE2 was identified as the chief virus receptor utilized to gain entry into the host cell, though other attachment factors have also been proposed (Jeffers et al. 2004; Li et al. 2003). A second human coronavirus, NL63, also uses ACE2 as a receptor for docking and entry (Pyrc et al. 2006). Isolation and in vitro expression of ACE2 molecules from various species such as mouse, civet and human have also helped elucidate important facets of epidemic and zoonotic SARS-CoV S and ACE2 interactions, virus host range expansion and the evolution of the epidemic strain (Li et al. 2006). ACE2 is expressed within lung epithelia, type I/II pneumocytes (the primary cellular targets of SARS-CoV) as well as within the intestinal epithelium, vascular endothelium, heart, kidney, and testis (Donoghue et al. 2000; Hamming et al. 2004). ACE2 and angiotensin I converting enzyme (ACE) are key regulators of the renin–angiotensin system (RAS), which helps control cardiovascular function by maintaining the body's blood pressure and electrolyte balance (Fig. 13.4) (Kuba et al. 2006b; Nicholls et al. 1998). ACE and ACE2 are metalloproteases with differing vasoactive peptide substrate specificities and as a result have disparate and antagonistic roles in maintaining physiologic homeostasis (Kuba et al. 2006b). ACE cleaves the peptide ANG I into ANG II, which has vasoconstrictive effects inducing hypertension while also inducing cell proliferation and fibrosis (Kuba et al. 2006b; Turner and Hooper 2002). In contrast, ACE2 processes ANG I into ANG 1-9 and further processes ANG II into the peptide ANG 1-7 which acts as a vasodilator while also being antiproliferative and apoptotic (Donoghue et al. 2000; Kuba et al. 2006b; Tipnis et al. 2000; Vickers et al. 2002). Current in vitro data suggests that ANG II ligation and signaling through angiotensin receptor 1a (AT1aR) can result in the production of proinflammatory cytokines (TNFα, IL-1β, IL-6, MCP-1, etc.), fibrosis, and cell proliferation (McAllister-Lucas et al. 2007). In vivo models of liver fibrosis or ALI support the above in vitro data, suggesting that ANG II exacerbates pathology and that this pathology is ameliorated by ACE2-related signals. In an acid aspiration model of ALI/ARDS, Imai et al. demonstrated that ACE2 protected mice from injury while ACE, ANG II and AT1aR promoted disease pathology (Imai et al. 2005). Similarly, Herath et al. demonstrated that ACE2 and ANG 1-7 counteracted the detrimental

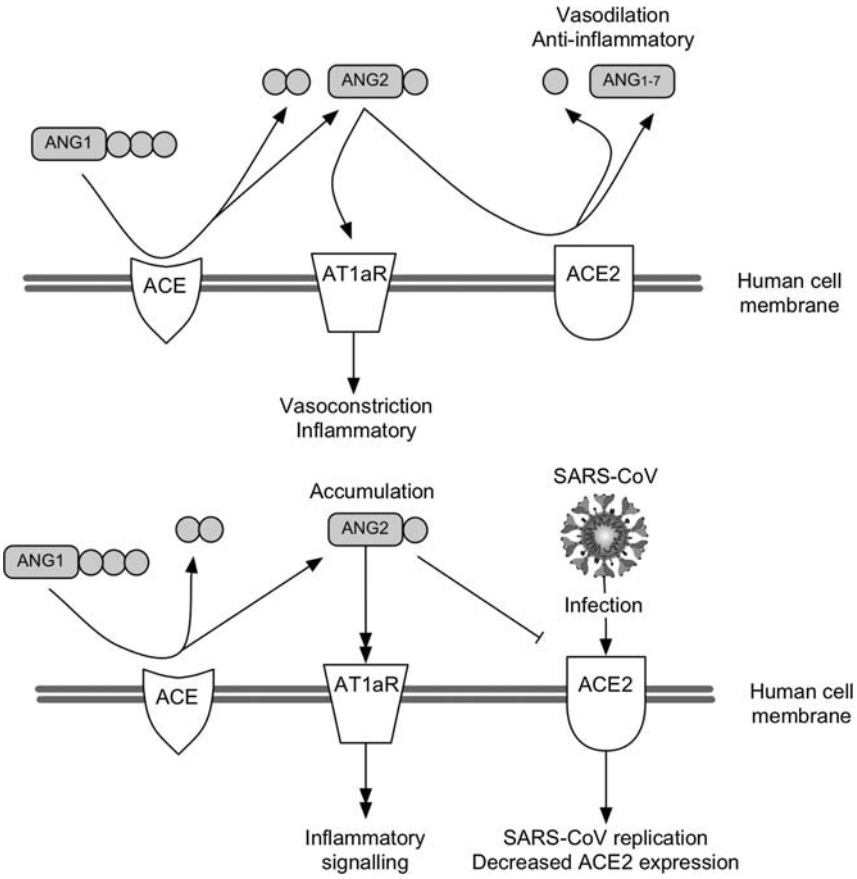



**Fig. 13.4** The SARS-CoV receptor, angiotensin I converting enzyme 2 (ACE2), in virus entry and pathogenesis. (**a**) ACE2 and angiotensin I converting enzyme (ACE) are key regulators of the renin–angiotensin system (RAS), which helps control cardiovascular function by maintaining the body's blood pressure and electrolyte balance. Current in vitro data suggests that ANG II ligation and signaling through angiotensin receptor 1a (AT1aR) can result in the production of proinflammatory cytokines (TNFα, IL-1β, IL-6, MCP-1, etc.), fibrosis, and cell proliferation. (**b**) Infection of the lung by SARS-CoV disrupts RAS homeostasis. Current data suggests that SARS-CoV infection or SARS S protein decreases levels of ACE2 within the lung, thereby removing a key regulator and processor of the proinflammatory ANG II peptide whose excess contributes to more severe disease

effects of ANG II in liver disease in rats (Herath et al. 2007). These data suggest a duality of RAS contributing to both homeostasis and immunopathology.

Perhaps the most interesting facet of the SARS-CoV and ACE2 relationship resides in the possible effect of virus infection on the local pulmonary disruption of RAS homeostasis (Fig. 13.4). Within a mouse model of SARS-CoV replication, Kuba et al. demonstrated that SARS-CoV infection diminished levels of ACE2 within the lung (Kuba et al. 2005). Recombinant SARS-CoV S protein delivered

intraperitoneally similarly reduced levels of ACE2 within the lung. Furthermore, within an acid aspiration model of acute lung injury (ALI), the administration of SARS S recombinant protein exacerbated ALI as measured by changes in lung elastance and the accumulation of edema within a 3 h period post acid injury (Kuba et al. 2005). It was proposed that SARS-CoV or SARS S decreased levels of ACE2 within the lung, thereby removing a key regulator and processor of the proinflammatory ANG II peptide whose excess contributed to more severe disease through proinflammatory signaling through AT1aR (Kuba et al. 2005). Recent data by Haga et al. suggest a mechanism for the downregulation of ACE2 in the lung and resultant increase in lung tissue damage. They found that the cleavage of ACE2 ectodomain on the cell surface was mediated by SARS-CoV S and TNF-α converting enzyme while the cytoplasmic tail of ACE2 simultaneously triggered the production of the tissue-destroying cytokine, TNF-α (Haga et al. 2008). Taken together, these data provide an interesting insight into possible RAS involvement in SARS-CoV pathogenesis and the progression of ALI to ARDS seen in more severe cases of SARS-CoV. The Kuba et al. model of acid aspiration-induced ALI is very acute (injury assessed within a 3 h window) compared with virus-induced lung injury where phenotypes evolve over many hours to days. Further, the acid aspiration model exists outside the context of virus infection and the induction of the innate and adaptive immune response, which were both found to contribute considerably to SARS-CoV pathogenesis in humans. Recently, an hACE2 transgenic mouse was created where hACE2 expression is targeted to epithelial cells via the K18 promoter (McCray et al. 2007). Although the K18 promoter targeted hACE2 expression to the lung epithelium, these mice also expressed a large amount of ACE2 in their central nervous system (CNS) epithelia. As such, infection of these mice with the epidemic strain, SARS Urbani, resulted in 100% mortality, most likely due to replication and infection within the CNS. Since CNS manifestations were not the chief pathological observation in human SARS-CoV infection, these data suggest that hACE2 transgenic mice under the control of nonlung cell-specific promoters may have limitations in determining pathways of SARS-CoV pathogenesis within the host. Although the K18 hACE2 mice most likely succumbed to infection of the CNS, the increased amounts of pulmonary ACE2 was not protective of lung pathology as predicted by the model presented by Kuba et al. Since K18 hACE2 transgenic mice are extremely susceptible to SARS-CoV infection, they may be useful as a highly stringent model to assess vaccine efficacy and challenge in the future. Given the interesting but conflicting data regarding RAS and SARS-CoV pathogenesis, future evaluation within current animal models of SARS-CoV pathogenesis may help resolve this discrepancy. It will be interesting to see if SARS-CoV infection of ACE- or AT1aR-deficient mice modulate the development of severe SARS-CoV disease. The use of commercially available drugs that block AT1aR (Telmisartan) or ACE (A0773) in the context of SARS-CoV infection may also provide interesting information on the involvement of the RAS system in SARS-CoV pathogenesis. Lastly, the uncoupling of ACE2 physiological function and SARS-CoV receptor function through the generation of catalytically inactive ACE2 “knock in” mice would allow for SARS-CoV infection, but

ACE2 cleavage of ANGII could not occur. One would predict that the infection of these catalytically inactive ACE2 mice would experience more severe disease due to the absence of the protective ACE2 metabolism of the proinflammatory ANGII.

## 13.3 SARS-CoV Therapeutic Design

It is clear that vaccination and passive immunization technologies are among the most important public health interventions in the past 200 years contributing to the complete eradication of smallpox (Marasco and Sui 2007; Plotkin 1999). Though vaccination campaigns have eradicated polio and measles in developed nations, these diseases and other vaccine-preventable diseases continue to plague developing nations (WHO 2008a, 2008b, 2008c). Rapidly and newly emerging infectious diseases like SARS-CoV provide unpredictable scenarios for the field of vaccinology, where diseases never before seen in human populations arise and spread rapidly while reagents necessary for vaccine development do not yet exist. Emerging viruses arising from zoonotic pools are especially problematic, as vaccines and therapeutics targeted against previously evolved strains might not function against strains associated with contemporary outbreaks of disease. As seen with the SARS-CoV epidemic, isolation of the virus allowed for the rapid generation of "killed," DNA and viral vectored vaccines within a year of the start of the epidemic (Sui et al. 2004; Tang et al. 2004). Given the sequence diversity in bat SARS-CoV reservoirs, it is likely that these killed vaccines will fail against newly emerged strains that arise in the future, especially since antisera directed against bat S glycoproteins do not neutralize human epidemic strains (Becker et al. 2008). Like vaccination, the practice of passive immunization to prevent infection or curtail established disease was first shown by Robert Koch in the late 1800s, when he demonstrated that sheep antisera against diptheria toxin could protect against death in humans (Marasco and Sui 2007). More recently, technologies like phage display and memory B cell immortalization have been developed to produce sufficient quantities of human monoclonal antibodies (hu-mABs) directed against specific viral antigens (Marasco and Sui 2007). These technologies allowed for the rapid development of neutralizing hu-mABs directed against SARS-CoV within a year of the beginning of the epidemic (Sui et al. 2004). We will discuss the problems associated with SARS-CoV vaccination and passive immunization therapies, which fall into three categories that include (a) SARS-CoV antigenic variation and therapy efficacy, (b) the complications of immunosenescence and SARS-CoV vaccine efficacy, and (c) SARS-CoV vaccine immunopotentiation of lung pathology.

### *13.3.1 SARS-CoV Antigenic Variation and Therapy Efficacy*

Within humans infected by the SARS-CoV, multiple antigens were targeted by the adaptive immune response. Although T cell responses directed against nonstructural replicase proteins have been measured in convalescent patients, the majority

of both T and B cell responses were directed against structural proteins (Li et al. 2008; Qiu et al. 2005; Yang et al. 2007). In a study exploring T cell responses in SARS-CoV patients by Li et al., the most frequently targeted peptides resided within the S and ORF 3a proteins, with lesser responses to E, M, and N proteins (Li et al. 2008). In studies evaluating the humoral response to SARS antigens in patient samples, antibody responses to S, ORF 3a, N and ORF9b were measured but only antibodies targeting the S protein were capable of neutralization (Qiu et al. 2005). Most studies demonstrate that only antibodies directed against the S glycoprotein are capable of neutralizing SARS-CoV, although conflicting data regarding anti-ORF-3a antibody neutralization has also been reported (Akerstrom et al. 2006; Cameron et al. 2007; Qiu et al. 2005; Yount et al. 2005).

The body of work related to SARS-CoV vaccine development is astounding. Unfortunately, many studies only evaluate the immune response to antigen and fail to evaluate the vaccine efficacy through SARS-CoV virus challenge (Bai et al. 2008; Jin et al. 2005; Liu et al. 2005; Zhang et al. 2005). Inactivated whole virus and vectored SARS-CoV vaccine trials in a number of different animals models have demonstrated that the SARS-CoV spike glycoprotein (S) is the critical component of protective immunity and the passive transfer of SARS-CoV S-specific sera is sufficient to provide protection from infection and disease caused by a homologous SARS-CoV strain (Buchholz et al. 2004; Deming et al. 2006; He et al. 2006; Kapadia et al. 2005; Qin et al. 2006; Spruth et al. 2006; Subbarao et al. 2004; Wang et al. 2005; Yang et al. 2004; Zhou et al. 2005, 2007a). However, current animal models universally display a very acute SARS-CoV-like disease and most vaccines have only been evaluated in the context of virus replication without severe acute lung injury (Chu et al. 2008; Deming et al. 2006; Haagmans et al. 2004; Haagmans and Osterhaus 2006; Roberts et al. 2006, 2007; Roberts and Subbarao 2006; Subbarao and Roberts 2006). As such, these models may underrepresent the importance of the cell-mediated and humoral responses in controlling more prolonged infection and pathogenesis as seen in human cases of SARS-CoV. In fact, the development of a SARS-CoV animal model that recapitulates both acute and prolonged infection with the development of adaptive immunity would greatly benefit the study of SARS-CoV pathogenesis and SARS-CoV vaccine development. Nevertheless, several replication, mouse-adapted lethal, and age-related models of acute SARS-CoV pathogenesis now exist and are currently the most effective systems within which to assess vaccine and passive immunization efficacy (Deming et al. 2006; Roberts et al. 2007, 2005a, 2005b; Rockx et al. 2007).

### *13.3.2 Animal Models to Assess Passive Immunization Therapy Efficacy Against Divergent SARS-CoV Antigens*

Effective human monoclonal antibodies (hu-mAbs) can be utilized for the prophylactic or acute treatment of viral infections (Marasco and Sui 2007). Since the generation of effective vaccines and vaccine-induced immunity can be time-consuming, the production of broadly neutralizing hu-mAbs targeting emerging viral

diseases may be valuable for use in healthcare workers and vulnerable populations in emerging viral outbreak situations in immunologically naïve populations. The past emergence of SARS-CoV from zoonotic pools and the continued circulation of SARS-like viruses within bat populations provides the perfect situation for the development of hu-mAb therapies to protect against future emergence. Due to the unknown antigenic identity of future emergent SARS-CoV, we are presented with a difficult problem: which cross-neutralizing epitopes should be targeted by passive immunization therapies in order to effectively treat future emergence of SARS-CoV? As with vaccination, the most successful hu-mABs for passive immunization against SARS-CoV should broadly neutralize all current and future SARS-CoV strains. One of the first hu-mABs developed against SARS-CoV, 80R, was effective in neutralizing pseudovirus-bearing epidemic (Tor2) and civet (SZ3) S proteins but was not as effective against pseudovirus bearing the civet-like GD03 S in vitro (Sui et al. 2005). Using hu-mAB m396, Zhu et al. reported complete neutralization of SARS Urbani and SARS-CoV bearing GD03 S (icGD03-S) but was less effective at neutralizing the SARS-CoV bearing the SZ16-K479N S (icSZ16-S K479N, 4 log reduction in virus lung titer as compared to control hu-mAB) in passive transfer experiments in young BALB/c mice (Zhu et al. 2007). In contrast to the m396 antibody, Zhu et al. also reported complete protection from virus replication (day 2 post infection) against SARS Urbani, icGD03-S, and icSZ16-S K479N using similar doses of hu-mAB S230.15 (Zhu et al. 2007). Rockx et al. also demonstrated the cross-reactivity and potent neutralizing ability of hu-mAB S230.15, S227.14, and S109.8 in passive transfer studies in mice (Rockx et al. 2008). S230.15, S227.14, and S109.8 effectively neutralized SARS Urbani and recombinant SARS-CoV bearing the early epidemic phase GZ02 S, though all were slightly less efficacious against recombinant SARS-CoV bearing the civet HC/SZ/61/03 S glycoprotein (2 log reduction in virus lung titers on day 2 post infection as compared to control Ab) (Rockx et al. 2008). Though S230.15 and S227.14 did not protect against virus replication in HC/SZ/61/03-challenged mice, both antibodies protected Urbani-, GZ02-, and HC/SZ/61/03-nfected mice from clinical signs of disease and death (Rockx et al. 2008). These data highlight the importance of using more than one strain of SARS-CoV when evaluating SARS-CoV therapies, since the hu-mABs discussed above provided varying degrees of protection from replication depending on the SARS-CoV S variant that was employed in the in vitro or in vivo assay. Also, these data suggest that the complete abrogation of replication may not be necessary to protect from clinical signs of SARS-CoV disease. Importantly, escape mutants generated from hu-mAB S227.14, S230.15, and S109.8 were significantly attenuated in mice.

### *13.3.3 Animal Models to Assess Vaccine Immunization Therapy Efficacy Against Divergent SARS-CoV Antigens*

Since the epidemic strain may no longer exist in nature, vaccination with epidemic strain antigens followed by challenge with the epidemic strain may not be the most biologically and medically relevant design. Due to the complications of designing

a vaccine against future emergence of SARS-CoV whose antigenic identity is unknown, several difficult questions arise in the development of effective SARS-CoV therapies: Which SARS-CoV antigen or pool of antigens will provide the greatest degree of cross-protection if the vaccination is to prevent disease from future emergence of SARS-CoV? Which vaccine formulation will be effective in the elderly? Lastly, which SARS-CoV strain(s) should be employed as challenge virus to assess vaccine efficacy? We can begin to answer these questions within current animal models of SARS-CoV pathogenesis.

Several animal models (see Sect. 13.2.1) and common vaccine formulations have been utilized in SARS-CoV vaccine development and these data are summarized in Table 13.2. Major approaches to SARS-CoV vaccine platform development include whole killed, recombinant viral vector, DNA, live-attenuated SARS-CoV, and recombinant protein subunit vaccines (Bisht et al. 2004, 2005; Chen et al. 2005; Czub et al. 2005; Darnell et al. 2007; Deming et al. 2006; Du et al. 2007, 2008a, 2008b; Faber et al. 2005; Gai et al. 2008; He et al. 2004; Jin et al. 2005; Kapadia et al. 2005, 2008; Kobinger et al. 2007; Lamirande et al. 2008; Liniger et al. 2008; Martin et al. 2008; Qin et al. 2006; Qu et al. 2005; See et al. 2006, 2008; Spruth et al. 2006; Tsunetsugu-Yokota et al. 2007; Wang et al. 2005; Weingartl et al. 2004; Yang et al. 2004; Zhang et al. 2005; Zhou et al. 2005; Zhu et al. 2004). Ideally, animal models used to assess protective vaccine efficacy would display virus replication, pathology, morbidity, and mortality. Though all of the models utilized to assess vaccine efficacy demonstrate virus replication and lung manifestations of disease, very few demonstrate virus-induced morbidity and severe lung pathology, and none demonstrate mortality (Table 13.2). In turn, the more stringent animal models (mouse-adapted SARS-CoV, hACE2 transgenic mice, etc.) that demonstrate morbidity and mortality that are currently available need to be employed in order to provide a thorough evaluation of vaccine protective efficacy. Moreover, very few of the above vaccine studies assessed protective vaccine efficacy with a SARS-CoV virus challenge, without which the utility and success of the vaccine remains unknown. Furthermore, since future SARS-CoV emergence will most likely differ in antigenic identity as compared to epidemic strain (SARS Urbani), vaccine challenge strains should ideally be antigenically distinct from the vaccine antigen(s), thus allowing for the important assessment of vaccine cross-protection. To our knowledge, only two SARS-CoV vaccine studies to date have assessed protective efficacy using a heterologous challenge virus (Deming et al. 2006; Lamirande et al. 2008). Successful vaccination in aged populations is a necessary goal for SARS-CoV vaccine platforms and, unfortunately, only one study to date has focused on developing effective vaccines in this most vulnerable population (Deming et al. 2006) |This conundrum is discussed below.

### *13.3.4 SARS-CoV Vaccine Efficacy in Immunosenescent Populations*

As mentioned above, the immunosenescence that occurs with aging can hamper both the innate and adaptive immune responses whose collaboration is necessary

**Table 13.2** Examples of common approaches to SARS-CoV vaccine development

| Platform | Vaccine formulation(s) | Vaccine antigen(s) | Animal model | Metrics of vaccine efficacy | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | T cell response measured | Antibody response measured | Neutralizing antibody measured | Homologous or heterologous challenge | Virus replication measured | Morbidity | Mortality | References |
| Killed SARS-CoV | FI, UV, or β-propiolactone ± alum | Whole virus | Mouse | Yes | Yes | Yes | Homologous (2/7 studies) | Yes | No | No | Gai et al. (2008); Qu et al. (2005); See et al. (2006); Spruth et al. (2006); Tsunetsugu-Yokota et al. (2007); Xiong et al. (2004); Zhang et al. (2005) |
| | FI or β-propiolactone ± alum | Whole virus | Ferret | No | Yes | Yes | Homologous (2/2) | Yes | Yes | No | Darnell et al. (2007); See et al. (2008) |
| | β-propiolactone ± alum | Whole virus | Non-human primate | No | Yes | Yes | Homologous (2/2) | Yes | Yes | No | Qin et al. (2006); Zhou et al. (2005) |
| Recombinant viral vector | MVA, VRP, MV, RV, VSV, AAV | S or N | Mouse | Yes | Yes | Yes | Homologous (2/8) and heterologous (1/8, Deming et al.) | Yes | Yes (Deming et al.) | No | Bisht et al. (2004); Chen et al. (2005); Deming et al. (2006); Du et al., (2008); Faber et al. (2005); Kapadia et al. (2005); Kapadia et al. (2008); Liniger et al. (2008) |
| | Adenovirus, MVA | S or N | Ferret | No | Yes | Yes | Homologous (3/3) | Yes | Yes | No | Czub et al. (2005); Kobinger et al. (2007); Weingartl et al. (2004) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Adenovirus, MVA | S or N | Non-human primate | No | Yes | Yes | Homologous (1/2) | Yes | No | No | Chen et al. (2005); Kobinger et al. (2007) |
| DNA | DNA plasmid | S, N, M, E, +/− adjuvant | Mouse | Yes | Yes | Yes | Homologous (1/4) | Yes | No | No | Jin et al. (2005); Wang et al. (2005); Yang et al. (2004); Zhu et al. (2004) |
| | DNA plasmid | S | Human | Yes | Yes | Yes | No | No | No | No | Martin et al. (2008) |
| Live attenuated | SARS-CoV deleted for E | Whole virus | Hamster | No | Yes | Yes | Heterologous | Yes | Yes | No | Lamirande et al. (2008) |
| Subunit | SARS S RBD | Recombinant protein | Mouse | No | Yes | Yes | Homologous (1/2, Du et al.) | Yes (Du et al.) | No | No | Du et al. (2007); He et al. (2004) |
| | SARS S 14-762aa ± saponin or a Ribi adjuvant | Recombinant protein | Mouse | No | Yes | Yes | Homologous | Yes | No | No | Bisht et al. (2005) |

FI: Formalin inactivated; UV: Ultraviolet radiation; MVA: Modified vaccinia virus Ankara; VRP: Venezuelan equine encephalitis virus replicon particle; MV: Modified measles virus; RV: Modified rhabdovirus; VSV: Modified vesicular stomatitis virus; AAV: adeno-associated virus

for efficient vaccination. The SARS-CoV epidemic was particularly harsh on aged populations where mortality ranged between 25 and 55% in people over the age of 65 (Booth et al. 2003; Leung et al. 2004; Liang et al. 2004; Peiris et al. 2003a). If a SARS-CoV-like virus were to reemerge in the future, it would be imperative that current vaccination strategies were successful in the most vulnerable populations. Unfortunately, the successful vaccination of elderly populations is a difficult and unpredictable task due to immunosenescence (Bernstein et al. 1999, 1998; Eaton et al. 2004; Effros 2007; Goodwin et al. 2006; Goronzy et al. 2001; Gruver et al. 2007; Haynes and Swain 2006; Pawelec and Larbi 2008; Vallejo 2005; Vasto et al. 2006). Much of the research related to vaccination of the immunosenescent has been performed with influenza. Current models predict that influenza vaccine efficacy in elderly populations ranges from 17 to 53% while the vaccine in young adults is 70–90% effective and the discrepancy seems to be a result of senescent immune system malfunction on multiple levels (Goodwin et al. 2006). Defects in antigen presentation, T cell activation, and cytokine secretion affect the generation of effective adaptive immune system helper (T helper or Th) cells and effector (B cells and cytotoxic T cells) cells resulting in diminished vaccine efficacy (Eaton et al. 2004; Effros 2007; Fujihashi et al. 2000; Goodwin et al. 2006; Goronzy et al. 2001; Haynes and Swain 2006; McElhaney et al. 2005; Vallejo 2005; Wang et al. 1995). Current research suggests that some defects of the senescent immune system can be overcome through administration of cytokines (IL-2) or adjuvants (MF59, CpG DNA) during vaccination that effectively activate APCs/Th cells, thereby increasing the probability of generating appropriate effector cells required for successful vaccination (Haynes et al. 2004, 1999; Higgins et al. 1996; Pulendran and Ahmed 2006; Thompson et al. 2006). Since influenza, West Nile virus, and SARS-CoV infection all produce disproportionately more disease in the elderly, the development of successful vaccine strategies in the elderly has a broad public health application (Anonymous 1995; Leung et al. 2004; Murray et al. 2006).

As in SARS-CoV-infection of aged humans, the infection of aged mice with SARS Urbani resulted in more severe disease as compared to similar infection of young adult mice. In senescent mice, both virus replication and lung pathology was enhanced but the virus was eventually cleared, suggesting that components of the aged immune system were less effective at controlling virus replication. Though the senescent mouse model does not fully recapitulate SARS-CoV acute and extended cell-mediated pathogenesis seen in humans, it serves as a useful model to study the effects of immunosenescence on vaccine efficacy. In 2006, Deming et al. demonstrated that a Venezuelan equine encephalitis virus replicon particle expressing SARS Urbani S (VRP-S) vaccine provided complete protection from replication of a SARS-CoV bearing a zoonotic heterologous GD03 S, but protection was variable in senescent mice (Deming et al. 2006). Due to the lack of significant morbidity and mortality in the SARS-CoV replication models, previous vaccine studies were unable to assess protection from disease or death and could only speculate that diminishing virus replication would diminish disease. Nevertheless, heterogeneity between antigen and challenge virus provides a more stringent,

thorough, biologically and medically relevant model within which to assess vaccine efficacy. Therefore, employing an antigenically diverse panel of SARS-CoV antigens for vaccination coupled with the use of a similarly diverse lethal challenge virus panel may represent the most pertinent and relevant strategy for assessing vaccine efficacy (Fig. 13.3). Moreover, the robustness of the newly developed MA15 lethal BALB/c model would allow for the assessment of vaccines to induce protection from not only replication but also disease and mortality. Using VSV vectors expressing Urbani S (rVSV-S), Vogel et al. obtained similar results in aged mice where neutralization titers in vaccinated aged mice were low and did not provide protection from replication upon homologous SARS Urbani challenge (Vogel et al. 2007). Similar to the situation observed in humans, these data suggest that vaccination of young mice induces a robust and cross-protective IgG response while the IgG response in aged animals is depressed in both magnitude and cross-reactivity. As compared with young animals, our data indicate that aged mice have ~10–20-fold reduced neutralization titers against homologous viruses and 100–400-fold reduced titers against closely related heterologous viruses (Deming et al. 2006). The underlying mechanisms of vaccine failure in the elderly should evolve into the major focus for future SARS-CoV vaccine research.

### *13.3.5 SARS-CoV Vaccine Immunopotentiation*

Effective vaccination induces specific protective immunity that confers protection against future disease. Unfortunately, vaccination can sometimes exacerbate disease upon natural infection with the pathogen the vaccine was designed to protect against. This phenomenon of vaccine-induced immune pathology is often called "immunopotentiation" of disease (Werle et al. 1999). Both measles (MV) and RSV are paramyxovirus respiratory pathogens that cause significant morbidity and mortality in infants that might be prevented through the development of effective vaccines (Polack et al. 1999; Varga et al. 2001). In the 1960s, two infamous examples of immunopotentiation of disease surfaced with formalin-inactivated MV (FI-MV) and RSV (FI-RSV) vaccines (Polack et al. 1999; Varga et al. 2001). Infants with no prior exposure to RSV who were vaccinated with the FI-RSV vaccine developed virus-specific antibody but were not protected from subsequent natural RSV infection (Durbin and Durbin 2004). In fact, FI-RSV-vaccinated children infected with RSV suffered from enhanced RSV disease requiring hospitalization, and a few children died from infection (Durbin and Durbin 2004). The pathological hallmark of FI-RSV immunopotentiation was lung and peripheral eosinophilia, which is rarely seen in the natural course of RSV infection (Durbin and Durbin 2004; Varga et al. 2001). Similar to FI-RSV, protective immunity waned in infants shortly after vaccination with FI-MV and subsequent natural measles infection resulted in more severe disease with eosinophilia uncharacteristic of natural measles infection (Polack et al. 1999). Due to these severe vaccine-associated disease complications, both vaccines were

withdrawn and research efforts focused on the elucidation of the underlying molecular mechanisms of these vaccine-induced pathologies. Within mouse and nonhuman primate animal models, both FI-RSV and FI-MV were found to induce an atypical Th2 adaptive immune response not seen in natural infection (De Swart et al. 2002; Durbin and Durbin 2004; Polack et al. 1999; Varga et al. 2001). The effects of the Th2 responses generated by FI-RSV and FI-MV differ. FI-RSV vaccination induces a Th2 allergic immune response with T cells secreting cytokines (IL-13, IL-5) that upregulate the production of the potent eosinophil chemotactic molecule eotaxin (De Swart et al. 2002; Durbin and Durbin 2004; Varga et al. 2001). Natural infection of vaccinated individuals is thought to have been exacerbated by this atypical allergic immune response in the lung (De Swart et al. 2002; Durbin and Durbin 2004; Varga et al. 2001). Interestingly, similar results are achieved in macaques using formalin-inactivated human metapneumovirus vaccines (FI-hMPV) followed by hMPV challenge (de Swart et al. 2007). With FI-MV vaccination, non-human primate models suggest that the associated allergic Th2 response generated after MV challenge recruits eosinophils to sites of virus replication with disease pathology in part mediated by immune complex deposition (Polack et al. 1999).

Vaccine-induced immunopotentiation has also been observed in coronavirus with vaccinia virus vectored feline infectious peritonitis virus (FIPV) vaccines. Vennema et al. observed that vaccination with recombinant vaccinia virus expressing FIPV S (vFS) induced short-lived immunity in kittens (Vennema et al. 1990). When challenged, vFS-immunized animals suffered from much more severe disease than those receiving a control vaccine. vFS vaccine-induced immunopotentiation was suspected to be a result of antibody-dependent enhancement (ADE) of virus infection where subneutralizing antibody coating FIPV virions allowed for the entry and productive infection of cells (e.g., macrophages) not normally targeted during natural infection.

Vaccine-induced immunopotentiation of disease has also been shown with vectored vaccines expressing SARS-CoV N protein. Deming et al. demonstrated that mice vaccinated with VRP-based vaccines expressing the SARS-CoV N gene were not protected from infection and developed enhanced lung immunopathology upon challenge with eosinophilia not seen in control mice, and these data have recently been confirmed by Yasui et al. (Deming et al. 2006; Yasui et al. 2008). These data suggest that the N protein not only fails to provide protection from disease in these acute replication models, it also promotes enhanced disease pathogenesis in the lung. The evaluation of several formalin-inactivated SARS-CoV vaccines suggest these vaccines primarily induce a Th2 response while natural SARS-CoV infection of humans induces primarily a Th1 response (Spruth et al. 2006; Tsunetsugu-Yokota et al. 2007; Wong et al. 2004). Given the data from both FI-RSV and FI-MV vaccination where the alteration of the natural Th response induced more severe disease upon challenge, caution should be used in developing vectored vaccines containing SARS-CoV N or formalin-inactivated SARS-CoV vaccines which may promote rather than prevent disease. In support of this viewpoint, we have shown that killed SARS vaccines in the presence or

absence of alum induce extensive lung pathology, especially in the lungs of aged animals inoculated with a heterologous lethal challenge viruses (Deming et al. 2009, in preparation).

## 13.4 Conclusion

Due to the ever-increasing human population, increasing wild-life habitat destruction for human inhabitation, the demand for exotic animals for food, and the inability of humans to control or successfully track zoonotic diseases in wild animal populations, the emergence of novel viral pathogens from zoonotic pools will continue to threaten human global public health. The development of antiviral therapies against viral pathogens that might emerge in the future is a difficult multifaceted problem, but it is critical for improving global health. SARS-CoV was the first significant emerging virus of the twenty-first century. The availability of reverse genetics, time-ordered sequence variation of animal and human strains, robust availability of biochemical reagents, and age-related animal models provide a unique opportunity to study many basic aspects of novel virus emergence and antigenic diversity, pathogenesis, antiviral therapy development, and vaccine immunopotentiation of disease. As SARS-CoV vaccines must provide broad protection against the larger zoonotic pool, successful vaccine strategies may provide a template for developing broadly reactive vaccines against other emerging viruses, like filoviruses, Nipah virus, NL63, HKU1, and avian influenza viruses. Importantly, SARS-CoV pathogenesis is exacerbated in the immunosenescent, a population that suffers a disproportionate disease burden from other emerging viruses. Through the use of aged models of SARS-CoV pathogenesis and vaccine efficacy, the immunological deficiencies of the aged immune system and/or the variables required for successful vaccination may be elucidated. These data may be applied to improve vaccines for other viral pathogens that cause a disproportionate disease burden in vulnerable populations like the elderly (West Nile virus, influenza, norovirus, SARS-CoV, RSV, etc.). In the past, the use of whole killed vaccines for vaccination has been successful in preventing disease but has also contributed to immunopotentiation of disease, and the mechanisms for this exacerbation of disease are not completely understood. Uncovering the mechanisms of SARS-CoV nucleocapsid-induced immunopotentiation may reveal common host pathways with other vaccine formulations (e.g., FI-RSV, FI-MV) that mediate vaccine-related pathologies. Alternatively, the unique genetic differences between coronaviruses and paramyxoviruses may reveal entirely new pathways for virus–host interactions that potentiate vaccine-induced immune pathology. Thus, current models of SARS-CoV pathogenesis can be employed to study the many difficult problems associated with the development of effective therapies for emerging pathogens, and future studies may provide the solutions that will prepare us for future SARS-CoV emergence or the emergence of yet unknown viral pathogens.

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