there may be no added advantage to use of these animals for evaluation of SARS vaccine efficacy (44,45).

Golden Syrian hamsters support efficient and prolonged (10–14 days) replication of SARS-CoV in the respiratory tract associated with decreased activity, pneumonitis, and pulmonary consolidation (46) permitting evaluation of several objective criteria. Ferrets also support replication of SARS-CoV in the respiratory tract following intranasal (IN) inoculation (47). Pulmonary virus replication is associated with histopathologic findings (47) but the extent of associated clinical symptoms is controversial (48,49). Cynomolgus and rhesus macaques, African green monkeys, and common marmosets have all been experimentally infected with SARS-CoV (50–55). The virus can be recovered from the lungs and is associated with histopathologic findings (52,53), but the extent of associated clinical symptoms is very variable and the virus is cleared quickly (53). Although some features of the disease in nonhuman primates are similar to what was seen in SARS cases in humans (50,54,55), nonhuman primates do not replicate SARS in humans faithfully (51,53), and the cost of studies in monkeys severely limits the size of experimental groups (50–55). Therefore, studies in nonhuman primates should be based on larger experiments in small animals and should be designed to answer specific questions.

ANTIBODY DEPENDENT ENHANCEMENT IN VITRO AND IN VIVO

One of the major safety concerns about a SARS-CoV vaccine is regarding the development of antibody dependent enhancement (ADE) of disease. ADE has been associated with many different viruses. It occurs when a virus-antibody complex interacts with Fc receptors (FcRs) or complement to trigger virus uptake or alternatively, when antibodies induce conformational changes in envelope glycoproteins that are required for virus-cell membrane fusion (56).

Initial concerns about ADE following SARS-CoV vaccines were based on the observation that accelerated and enhanced disease occurred on reexposure to feline infectious peritonitis virus (FIPV) in seropositive cats. Infection of macrophages by FIPV is believed to be important in the pathogenesis of accelerated disease; the enhanced disease was mediated by enhanced entry of FIPV into macrophages through anti-S antibodies binding to FcR expressed on the macrophages (57,58).

Additional concerns about ADE associated with SARS-CoV are related to the following four reports: (*i*) Entry of pseudotyped lentiviruses expressing the S protein of a SARS-CoV isolated from a palm civet into a human renal epithelial cell line was enhanced by human S–specific neutralizing antibodies (59). (*ii*) Although SARS-CoV primarily infects epithelial cells in the lungs of mice, hamsters, and nonhuman primates, there is evidence of infection in some macrophages in nonhuman primates as well (53). (*iii*) Sera from mice and hamsters immunized with a recombinant native full-length trimeric spike protein vaccine and convalescent human sera showed a 100- to 1000-fold increase in virus entry into FcγRII positive, ACE-2-negative human B cells; this was mediated by the Fc region of the antibody and the FcγRII receptor (56). (*iv*) When ferrets that were immunized intraperitoneally with a modified vaccinia virus expressing the S or N protein of SARS-CoV were challenged with SARS-CoV, they were not protected. The ferrets were sacrificed on 27 to 29 days later, and all the animals had periportal and panlobular hepatitis with the most severe hepatitis with focal liver cell necrosis seen in animals that had been vaccinated with the modified vaccinia virus Ankara (MVA)-S vaccine (48,60).

Although these reports raise some questions, the first report involves an assay of entry of a pseudotyped virus but has not been extended to use of an authentic SARS-CoV or the presence of an in vivo correlate of the enhanced entry of the pseudotyped virus. The report of enhanced entry into human B cell lines was not seen in mouse macrophages, despite the presence of FcγRII and B cells were only occasionally infected in SARS patients. Also, the trimeric spike vaccine elicited a protective immune response in vivo in hamsters (56). Neither of these studies is consistent with the ADE seen following FIPV vaccine. In the case of the MVA-S vaccine-induced hepatitis in ferrets, all the ferrets developed hepatitis and viral antigen was not detected in the liver. Also, MVA-S vaccines evaluated by two other groups of investigators were efficacious. Additionally, in a separate study in which ferrets were immunized with a weak inactivated vaccine, there was no evidence of enhanced disease (49).

There are several studies in experimental animals in which sub-neutralizing levels of antibodies were present when the animals were experimentally infected with SARS-CoV, but the animals did not show signs of enhanced viral replication or disease (5,49,61,62). On the basis of these observations, it is reasonable to conclude that SARS-CoV vaccines are not associated with ADE, as described with FIPV. However, as discussed below, there is reason to be cautious about vaccines that contain the N protein of SARS-CoV. An attenuated Venezuelan equine encephalitis virus expressing the N protein of SARS-CoV (VRP-N) failed to protect mice from homologous and heterologous SARS-CoV challenge and resulted in enhanced immunopathology with eosinophilic infiltrates in the lungs of mice after challenge. This pathology presented at day 4, peaked at day 7, and persisted through day 14, and was likely mediated by cellular immune responses in the absence of effective neutralizing antibody response (63).

PASSIVE IMMUNIZATION

From previous studies of other coronaviruses and from early investigations of SARS in animal models, it became clear that neutralizing antibodies generated following infection are directed at the spike protein, and neutralizing antibodies could prevent replication of the SARS coronavirus in the lungs of mice. Convalescent plasma was administered to patients with SARS without adverse effects (64,65) but the benefit of this treatment strategy cannot be assessed because it was not a controlled clinical trial. MAbs represent an ideal alternative to hyperimmune sera. Strategies for generation of MAbs for use in humans include: (*i*) humanization of murine MAbs through protein engineering, (*ii*) selection of antibodies from phage-display libraries of human antibody fragments, and (*iii*) immunization of transgenic mice carrying human immunoglobulin loci followed by production of MAbs using the hybridoma technology. The MAbs that had potent in vitro neutralizing activity were able to prevent infection in mice (37,66,67), hamsters (68), and ferrets (69), and at least two human MAbs have been identified that cross-react with human SARS-CoV and zoonotic isolates in vitro and in vivo (70). Postexposure treatment with a MAb alleviated virus burden and the degree of associated pathology, including interstitial pneumonitis and consolidation in the hamster model (68).