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Animal models and antibody assays for evaluating candidate SARS vaccines: Summary of a technical meeting 25–26 August 2005, London, UK

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Abstract

Severe acute respiratory syndrome (SARS) emerged in the Guangdong province of China in late 2002 and spread to 29 countries. By the end of the outbreak in July 2003, the CDC and WHO reported 8437 cases with a 9.6% case fatality rate. The disease was caused by a previously unrecognized coronavirus, SARS-CoV. Drawing on experience with animal coronavirus vaccines, several vaccine candidates have been developed and evaluated in pre-clinical trials. Available data suggest that vaccines should be based on the the 180 kDa viral spike protein, S, the only significant neutralization antigen capable of inducing protective immune responses in animals. In the absence of clinical cases of SARS, candidate vaccines should be evaluated for efficacy in animal models, and although it is uncertain whether the United States Food and Drug Administration's "animal rule" would apply to licensure of a SARS vaccine, it is important to develop standardized animal models and immunological assays in preparation for this eventuality. This report summarizes the recommendations from a WHO Technical Meeting on Animal Models and Antibody Assays for Evaluating Candidate SARS Vaccines held on 25–26 August 2005 in South Mimms, UK, provides guidance on the use of animal models, and outlines the steps to develop standard reagents and assays for immunological evaluation of candidate SARS vaccines.
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1. Introduction

Severe acute respiratory syndrome (SARS) is a severe respiratory illness caused by the SARS coronavirus (SARS-CoV) [1]. The disease emerged in the Guangdong province of China in late 2002 [2] and spread to 29 countries mostly within Asia, although Europe and North America were also affected, notably Toronto, Canada. The epidemic was finally controlled by July 2003 through strict implementation of quarantine and isolation procedures, culling of wild animals in live exotic animal markets and international collaboration under the coordination of WHO [3]. By the end of the outbreak, the CDC and WHO reported 8098 cases with a 9.6% case fatality rate [4]. Only sporadic cases have been reported since then, mainly linked to laboratory exposure.

A novel virus was isolated in Vero cells from the respiratory secretions from a patient with SARS [5,6]. Sequence analysis showed that it was a previously unrecognized coronavirus, SARS-CoV [7–9]. Serological and genetic evidence supports a zoonotic origin of SARS-CoV [10,11]. Animal traders working with masked palm civets in China had high

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prevalence for SARS-CoV antibody, although they had no history of SARS-like disease. SARS-CoV-like viruses that were isolated from civets and raccoon dogs had more than 99% homology with human SARS-CoV, with major differences found in ORF8, whose deletion has been suggested to represent a sign of adaptation to humans [12]. Only four amino acid residues in the receptor glycoprotein ACE2-binding domain of the viral spike protein differ between the human epidemic SARS-CoV strains and civet strains, but they cause more than a 1000-fold difference in binding affinity to the ACE2 molecule [13,14]. Although a high prevalence of SARS-like coronaviruses were found in Chinese horseshoe bats [15,16], their great genetic diversity makes it difficult to identify which one might be the ancestor of SARS-CoV and to decide with certainty whether bats indeed are the animal reservoir of the virus.

SARS-CoV infection exhibits a wide clinical course characterized mostly by fever, dyspnea, lymphopenia and lower respiratory infection, often with concurrent gastrointestinal symptoms including diarrhea [17,18]. Pathology in SARS patients has been associated with diffuse alveolar damage, epithelial cell proliferation and multinucleated giant cell infiltrates of epithelial or macrophage origin, suggestive of syncytium-like formation in the lung. The virus can be recovered from peripheral blood mononuclear cells, respiratory secretions, stools, urine and even sweat (for a review, see [19]). SARS vaccine development efforts were initiated very rapidly after the identification of the etiologic agent, even though the immune correlates of protection were not known. Research efforts to identify protective antigens and to develop animal models were undertaken in parallel with efforts to develop candidate vaccines [20], drawing on experience with animal coronavirus vaccines and using several vaccine strategies, including inactivated virus vaccines, purified subunit vaccines, plasmid DNA and viral vector-based vaccines as well as virus-like particles. Much effort has been made to identify appropriate animal models for SARS-CoV replication and pathogenesis. Several research groups have shown that mice [21,22], ferrets [23], hamsters [24] and nonhuman primates [25–30] support replication of SARS-CoV with varying degrees of associated disease.

These animal models were used for the evaluation of candidate vaccines, and the common conclusion that has emerged from the evaluation of several vaccines is that the 180 kDa viral spike protein, S, is the only significant neutralization antigen [31–34] and the only one to elicit protective immunity in animal models [21,35–40]. The S protein can be divided into two domains by analogy with other coronavirus spike proteins: an N-terminal S1 domain, which contains the receptor-binding site and neutralization epitopes and a C-terminal S2 domain which forms the membrane-anchored stalk region and contains a putative fusion peptide followed by two heptad repeats predicted to form a six-helix coiled-coil bundle [41].

In the absence of clinical cases of SARS, candidate vaccines will have to be evaluated for efficacy in animal models. The United States FDA "animal rule" states that, when efficacy studies in humans are not feasible, vaccines may be approved based on animal data alone, provided the pathophysiological mechanism of the disease is reasonably well-understood as is its prevention or reduction by the vaccine. Moreover, the protective effect of the vaccine should be demonstrated in more than one animal species expected to react with a response predictive for humans. The endpoint of animal studies should be clearly related to the desired benefit in humans (i.e. enhancement of survival or reduction in major morbidity), and the data generated should allow selection of an effective dose in humans. At the present time it is uncertain whether the "animal rule" would apply to licensure of a SARS vaccine. However, it is important to develop standardized animal models and immunological assays in preparation for this eventuality.

Scientists at the WHO Technical Meeting on Animal Models and Antibody Assays for Evaluating Candidate SARS Vaccines held on 25–26 August 2005 in South Mimms, UK, discussed many aspects of research pertaining to the use of animal models in vaccine development including available animal models, suitability of the various models, correlates of protection, critical components of potential vaccines, and the potential for disease enhancement in vaccinated animals following exposure to SARS-CoV. In addition, standardization of antibody assays and the establishment of a WHO International Standard for SARS-CoV antibody were also discussed. This report endeavors to summarize the recommendations from this meeting, based on consensus agreement. Recommendations for use of each animal model are given in Section 2 below. Correlates of protection, an overview of vaccine development, and observations pertaining to potential disease enhancement are summarized in the following Sections 3–6.

2. Animal models for evaluation of SARS-CoV vaccines and antiviral treatments

2.1. General considerations

In selecting animal models for vaccine evaluation, it is important to remember the principle underlying the so called "animal rule", where data from more than one animal species is often required: each animal species should contribute something different to our understanding of disease and protection. At this time, no single model offers a direct reproduction of what was seen in humans with SARS. Pathology (including pneumonitis, alveolar edema, and diffuse alveolar damage (DAD)) in humans is probably the most difficult element to reproduce in an animal model. Attention also should be given to the reduction of viral shedding because this would likely correlate with decreased risk of further spread of the disease among humans. In using animal models to study aspects of SARS-CoV infection, it must be emphasized that the kinetics of viral replication and appearance and resolu-

tion of pathological findings are much more rapid in animal models than in humans.

Whichever animal model is employed, special consideration should be given to the presence of co-existing pathogens, the age of the animals and the route(s) of infection. A sufficient number of time points and large enough number of animals should be used to allow statistical evaluation. The strain of SARS-CoV used also could be of importance.

It should be emphasized that different species may prove useful for studying different aspects of SARS-CoV. Whereas vaccines or antivirals may be addressed in many models, pathogenesis is best evaluated in those animal models for which immunological tools and reagents are available for detailed analysis of the immune response to the vaccine. This includes inbred mice and rhesus and cynomolgus macaques. It may actually be worthwhile to enhance the virulence of a SARS-CoV isolate by serial passages in an animal model to produce a challenge virus stock for vaccine studies that would elicit more reproducible disease in the animals. If a highly virulent host-adapted virus were to become available, such as a mouse-adapted or a monkey-adapted SARS-CoV strain, demonstration of the capacity of vaccines to protect against challenge with these more virulent strains would provide an almost ideal animal model.

Different models may also need to be employed to evaluate pathogenesis versus immunogenicity. For pathogenesis studies in animal models, mortality is not required as a readout. It would be ideal to develop animal models with comparable levels of mortality to that seen in humans (∼10% overall), including the increased mortality at increased age (∼50% > age 60). The optimal result would be to demonstrate efficacy of vaccines or antivirals in SARS-CoV animal models that mimic human morbidity and mortality and that show protection without vaccine-associated immunopathology.

2.2. *Recommended animal models*

2.2.1. *Mice*

Inbred mouse strains (BALB/c, C57BL/6, 129SvEv, STAT1−/−) support SARS-CoV replication and can develop pneumonitis (129S), but pneumonia and clinical symptoms are only observed in older BALB/c mice [24]. The mouse model is suitable for immunogenicity and efficacy studies of vaccines.

Prolonged viral replication, dissemination of virus to liver and spleen and accompanying pathology are seen in STAT1−/− mice; these mice, therefore, also are suitable for studies of pathogenesis and evaluation of antiviral drugs. Specific pathogen-free (SPF) animals must be used. Their age can be either 4–8 weeks or over 12 months and should be specified. The number of animals included must be sufficient for statistical analysis, and should include mock-infected controls.

A variety of SARS-CoV strains has been tested in mice, including Urbani, Frankfurt, HKU-39849, Tor-2, and the mouse-adapted SARS-CoV strain MA-15. These were inoculated by the intranasal (IN) route under light anesthesia [21,42], using a dose of 10^5 $TCID_{50}$ in 50 μL/mouse. Critical time points for specimen collections are days 2 (peak titer) and 5 post-infection (p.i.) for quantitative virology, days 3 and 5 p.i. for the study of interstitial pneumonitis and DAD in aged mice (inflate lungs with 10% neutral-buffered formalin), and day 9 for resolution.

2.2.2. *Hamsters*

Golden Syrian hamsters are an excellent animal model because they demonstrate high levels of SARS-CoV replication and develop pneumonitis. Hamsters are suitable for vaccine efficacy, immunoprophylaxis and treatment studies [24]. In contrast with BALB/c mice, in which the virus is detected only in the respiratory tract and is cleared by day 5 p.i., hamsters demonstrate a longer duration of viral shedding from the upper respiratory tract, some transient viremia, spread of the virus to liver and spleen and, most significantly, inflammation of respiratory tissues [21,24].

SPF animals older than 5 weeks of age should be used in sufficient number for statistical analysis and study design should include mock-infected animals. The animals can be housed in pairs or by three if the experiment is to last less than 4–6 weeks. Reserve space should be available to separate the animals in case of fights. Males and littermates tend to fight less.

Virus strains that have been tested in hamsters are Urbani, Frankfurt and HKU-39849. Virus should be administered by the IN route under light anesthesia, using a dose of 10^3 $TCID_{50}$ in 100 μL/hamster.

As an outcome of efficacy studies, quantitative virology should be preferred over quantitative PCR. For pathology studies, one can grade pathology as none, mild, moderate or severe as per Roberts et al. [24]. Critical time points for specimen collection are days 2 or 3 p.i. (peak viral titer) and 7 (clearance) for quantitative virology studies; and days 5 p.i. (consolidation and pneumonitis) and 14 or 21 (resolution) for pathology studies (lung).

2.2.3. *Ferrets*

There is evidence from one study that ferrets support SARS-CoV replication and develop pulmonary lesions [23] but according to another study, the animals remain asymptomatic, in the presence of SARS-CoV replication [38]. In view of these conflicting data, the ferret model needs to be further studied to determine its optimal utility for vaccine efficacy and immune prophylaxis studies. Additional studies are needed to define the extent of biological variability of the model and the possible role of co-pathogens that may contribute to the variability observed between different laboratories.

Animals aged 6 months or older should be used. Although not well documented, more consistent viral replication, pathology and clinical symptoms seem to be observed in older animals. The animals should be screened for viral co-

pathogens: Aleutian mink disease, respiratory viruses, hepatitis viruses, and others. The route of inoculation may be IN or IT, but not IV. The dose of virus (strains Tor-2, HKU-39849) sufficient to ensure reproducibility of infection in all animals is likely to be 10^5pfu or more/ferret.

Again, quantitative virology is preferred over qRT-PCR. For pathology studies, the same recommendations apply as for nonhuman primate studies (see below): slides should be shared between pathologists to develop a scoring system. Regarding specimen collection of respiratory tissues, further studies are needed to establish how much variation occurs in samples from different lobes of the lungs. Critical time points are day 3 p.i. for quantitative virology and days 4–5 p.i. for pathology studies (pneumonitis).

2.2.4. Nonhuman primates (NHP)

NHPs support SARS-CoV replication and develop pneumonitis with a variable degree of clinical symptoms depending upon the species employed. No single NHP species is preferred at this time. The number of animals in a given study needs to be large enough to account for animal-to-animal variability: a sample of 4 or 5 animals is not sufficient. In view of the cost of the experiments, challenge studies should be limited to those vaccine candidates that are most promising, using larger sample sizes (10–12 animals/group) and avoiding animals with free-range periods in life if possible. Immunological responses are best studied in species for which microarrays and reagents for identifying immune components are available, such as rhesus or cynomolgus macaques. However, the limited viral replication observed in cynomolgus macaques might be a disadvantage in selecting this species for studies. Other recommended NHP species are the common marmoset and African green monkeys (AGMs, *Chlorocebus aethiops sabeus*). The country of origin may play an important role and should be specified, e.g. Philippines (cynomolgus macaques, *Macaca fascicularis*); China (rhesus, *Macaca mulatta*); Brazil (marmosets, *Callithrix jacchus*), etc.

Prior to the experiment, the animals should be housed indoor to limit exposure to potential co-pathogens. They should be screened for parasites (*Strongyloides*, *Pneumonyssus simicola* (lung mites)) and for possible viral co-pathogens (retroviruses, respiratory viruses, adenoviruses). The SARS-CoV strains tested in NHP models are HKU-39849 (cynos), PUMC (rhesus) and Urbani (marmosets and AGMs). These were inoculated by the respiratory route (IN, IT) at a dose of 10^6pfu or more/NHP.

Here again, quantitative virology is preferred over qRT-PCR. For pathology studies, it would be an obvious advantage that laboratories share pathology slides for review by different pathologists in order to develop a scoring system. Specimens of respiratory tissues should be collected, but further studies are needed to establish how much variation occurs in samples from different lobes of the lungs, as was done and reported for African green monkeys (AGMs) [28]. Critical time points are days 2–4 p.i. for quantitative virology in cynomolgus macaques and AGMs, and later than day 4 p.i. for rhesus macaques of Chinese origin. For collection of tissues for histopathological analyses, days 2–4 p.i. are optimal for cynomolgus macaques and AGMs, and later than day 4 p.i. for rhesus macaques. Due to limitations of immunological reagents (including microarray assays now available), research may be limited to rhesus and cynomolgus macaques.

2.2.5. Other animal models: civets, rats, guinea pigs, wild voles

Data on other animal models are insufficient for consideration for use in SARS-CoV vaccine and antiviral evaluations [43–45]. Any additional model other than the four listed above (Section 2.2.1 to Section 2.2.4) would require thorough characterization including viral replication data and histopathological analysis of SARS-CoV-infected and mock-infected animals of the same age and gender. Viral replication and histopathological data in any new animal model should be reminiscent of at least some aspect of SARS in humans.

3. Correlates of protection

Although all the correlates of protection from SARS associated disease have not been identified in human infections, neutralizing antibodies are present in convalescent human serum. Antibodies to SARS-CoV spike (S) protein have been shown to prevent virus entry and neutralize virus infectivity *in vitro* [32,46]. Prophylactically administered monoclonal antibodies and passively transferred SARS-CoV hyper-immune sera have been shown to prevent SARS-CoV infection and associated disease following SARS-CoV challenge of naive mice and hamsters [21,34,47–49]. Monoclonal antibodies administered therapeutically (i.e. post-infection) also have been shown to limit viral replication and reduce associated disease in hamsters [50].

Although cell mediated immunity may have a protective role in viral clearance or resolution of disease, work in animal models shows that antibody alone is effective for prevention and treatment of SARS. Thus, mice immunized with live-recombinant vaccines expressing the SARS-CoV spike protein, using rabies virus [51], vesicular stomatitis virus (VSV) [52], adenovirus (Ad5) [27,53] or attenuated vaccinia virus MVA [36,38] as a vector, as well as mice immunized with DNA vaccines expressing the S gene [37,54], developed neutralizing antibodies to SARS-CoV and were protected against SARS-CoV challenge. Similar findings were reported after mucosal immunization of hamsters and AGMs using a bovine parainfluenza virus type 3 (BPIV3) vector expressing the SARS CoV S gene [33,39]. Several whole inactivated virus and recombinant protein candidate vaccines also have been developed and shown to elicit a neutralizing antibody response that provided protection against infectious challenge [55–60]. In addition, passively administered sera from vaccinated animals prevented SARS-CoV infection upon subsequent challenge of naïve mice, demonstrating that

antibodies induced by these vaccines did confer protection [37,52].

The neutralizing antibody titer that is necessary to achieve protection in humans exposed to SARS-CoV is, however, still not known.

It was recommended that when evaluating vaccine efficacy in future animal experiments, the challenge virus should be administered at two different time-points, once when post-immunization neutralizing antibody titers are high, and later when neutralizing antibody titers have waned or are low. It also was suggested that viral titers and pathology should be evaluated at two different time points. Specific times points for sample collection are given for each animal model in Section 2 above.

4. Disease enhancement

Previous observations of disease enhancement have been reported for human viral pathogens and shown to be due to antibody-mediated enhancement of virus entry (for reviews see [61,62]). 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 [63–65]. Aggravated FIP is apparently a result of enhanced viral entry into macrophages mediated by sub-neutralizing antibody levels [66]. Children vaccinated with inactivated respiratory syncytial virus (RSV) vaccines developed serious disease on subsequent exposure to RSV [67–69]. Individuals exposed to one of the four serotypes of Dengue virus developed severe disease when subsequently infected with a second, different serotype [70,71]. Enhanced disease following RSV vaccine or dengue infection occur by different mechanisms than FIPV. In view of such examples of enhanced disease following infection in a vaccinated host, there has been heightened concern that a similar phenomenon could occur with SARS-CoV vaccines. It was highly recommended, therefore, that known mechanisms of disease enhancement observed with other viruses and especially with other coronaviruses should be examined in SARS-CoV infections, especially in vaccinated animals. Although none of the studies to date have shown enhanced respiratory disease following SARS-CoV challenge in previously immunized animals, further studies in this area are warranted in view of some of the available *in vitro* data.

Antibodies against human SARS-CoV isolates were shown to enhance the entry of pseudo-typed viruses expressing the civet SARS-like CoV-spike protein into a human renal adenocarcinoma cell line (786-O). Enhancement was only demonstrated at the level of entry, but not of replication [72]. This phenomenon was seen with pseudo-typed lentiviruses expressing SARS-CoV spike protein of civet sequence specificity, but not with pseudo-typed viruses expressing spike proteins of human SARS-CoV isolates. It also was not observed with human isolates of SARS-CoV. The role of enhanced viral entry, as observed in these *in vitro* studies, has not been related to any known component of human disease or infection *in vivo*. However, given that SARS-CoV may replicate, albeit poorly, in human PBMCs [73,74], *in vitro* experiments looking for antibody-enhancement of SARS-CoV replication in human cells (e.g. macrophages and B-cells) should be performed.

Several groups have studied SARS-CoV infection in animals in the presence of neutralizing and sub-neutralizing levels of SARS-CoV anti-sera or anti SARS-CoV S-protein monoclonal antibodies, but no evidence of enhanced respiratory disease has been observed. However, foci of hepatic necrosis were noted following SARS-CoV challenge in MVA-SARS-S immunized ferrets [38]. Although these findings are worrisome, several questions were raised regarding the significance of the observation. The MVA-SARS-S vaccine used in these experiments was poorly immunogenic in the ferrets. The question of whether there could have been any co-pathogen in the animals was raised. It also would be important to know if the observed phenomenon depends on the MVA vector or on the animal model. It was strongly urged, therefore, that the experiment be repeated in ferrets. Additional experiments, in nonhuman primates and hamsters, looking for evidence of enhanced respiratory and hepatic diseases upon vaccination and challenge were also encouraged.

5. Standardization of immunological assays—a proposed International Standard for SARS-CoV antibody

Several candidate SARS vaccines that are at various stages of pre-clinical and clinical development are being developed worldwide. In China alone, three companies have been given regulatory approval for the clinical evaluation of a candidate SARS vaccine. It is important, therefore, to be able to compare data from each of the candidate vaccines, which, in turn, requires international standardization of the immunological assays used for the evaluation of these vaccines.

The accepted method of international standardization is to employ a WHO International Standard (IS), which allows comparison of results from different laboratories [75]. This is essential for establishing international requirements for vaccines, diagnostics or therapeutics. An IS is prepared from material bearing a close resemblance to the samples being assayed; the material is distributed in glass ampoules with high precision and reproducibility and then freeze dried. It is important that a sufficient number of ampoules (2000–3000) be prepared so as to provide for about 10 years of use, and that the activity of the contents remain stable over this period. The process of establishing a WHO IS involves an international collaborative study, in which the candidate IS is compared with other samples. If the results of the tests are suitable, the candidate IS assigned a provisional arbitrary unitage, a report is distributed for approval by study participants and

for eventual approval by the WHO Expert Committee on Biological Standardization (ECBS). The preparation, storage and distribution of over 90% of IS have been undertaken by the National Institute for Biological Standards and Control (NIBSC) at South Mimms (UK), which is a WHO Laboratory for Biological Standards.

NIBSC has developed in the past several WHO ISs for calibration of the antibody response against virus vaccines, including ISs for antibodies against Dengue, Hepatitis A virus (HAV), [76] Hepatitis B virus (HBV), Measles, [77] Polio, Rabies, [78] Rubella, and Smallpox. A candidate standard against Human Papilloma virus-16 (HPV-16) is under evaluation. The corresponding IS's were used for a variety of antibody assays including virus neutralisation (VN), haemagglutination-inhibition, single radial diffusion, enzyme-linked immunoassay and radio-immunoassay. Antibody ISs are most useful in epidemiological studies and in clinical trials. Their use allows correlates of immunity and potency requirements of prophylactic and therapeutic products to be expressed in International Units (IUs). Data from several collaborative studies demonstrate that use of an IS generally reduces the level of variability between assay results.

However, there may be problems in using ISs due to the complex array of antibody populations in each serum and the different sensitivity of different assay systems. Examples of potential problems can be found in HBV and Parvovirus B19 studies [79], which showed that different assay kits gave different results even when the IS was included in the assays. Another issue is the degree of antigenic homology between the viral antigen used for the preparation of the IS and the virus used in the assays. In a JEV collaborative study, a candidate antibody IS, which had been prepared from the sera of vaccinees immunized with an inactivated vaccine that was antigenically different from some of the viruses used in VN assays, demonstrated that the response to at least some inactivated vaccine is strain specific and the candidate IS was consequently not established by the WHO ECBS. Whether a panel of monoclonal antibodies to SARS-CoV could be used to prepare an IS is an attractive alternative which should be explored.

Significant progress with standardisation of SARS-CoV antibody assays had been made in China with the development of a national antibody standard. In order to develop the national standard, sera were collected from 20 convalescent SARS patients who were found to have SARS-CoV VN antibody titers ranging from undetectable to 1:203. One serum sample was selected for further evaluation based on cross-reactivity with four SARS-CoV strains and on Western blot analysis. This serum was freeze dried in 0.5 mL aliquots and was then assessed for stability by VN assays. The Chinese standard was assigned a VN titer of 52.7 with 95% confidence limits of 47.6–62.2.

A further important development in China was the preparation of human immunoglobulin for treatment of SARS patients. National guidelines have been prepared for collection of plasma, quality control testing and standardisation of assays. Three Chinese manufacturers have been licensed for preparation of SARS immunoglobulin. The source material was plasma from convalescent patients at more than 28 days after infection. All were in good health and their plasma tested negative for blood-borne agents. Plasma samples were processed by a combination of cold ethanol fractionation and ultrafiltration. In September 2003, three lots of IgG were produced and assayed by nationally-agreed procedures. The stock of immunoglobulin currently available is sufficient to treat 100 patients. Monoclonal antibodies have not yet been considered for treatment purposes in China.

The importance of assessing immunogenicity of candidate SARS-CoV vaccines using VN assays is well acknowledged, but the variety of VN tests in use is a significant problem since there is at this time no consensus on the most sensitive, specific, and reproducible assay system. It is therefore desirable to establish an antibody IS to serve as a basis of comparison in all VN assays. The most important activity at this time is to obtain a suitable source of antibody. A number of options can be considered, such as convalescent human sera, post-immunization human sera, monoclonal antibodies or hyperimmune animal sera. As an example, the availability of a suitable source of serum from convalescent patients in Hong Kong needs to be explored, although antibody levels in these individuals are probably quite low by now.

It also would be important that other assays than VN be included in the collaborative study, and that the impact of SARS-CoV strain variation be examined by using different SARS-CoV strains and/or sera with different specificities.

The Centralized Facility for AIDS Reagents (CFAR), which is based at NIBSC, could be a suitable model for a SARS-CoV repository [80]. The CFAR was established in 1989 to support AIDS vaccine research and it is now EU-funded [81]. There are currently 2000 reagents available including peptides, recombinant proteins, human sera/plasma, monoclonal and polyclonal antibodies, expression systems, cDNA clones and viruses. A comparison can be drawn between SARS-CoV and HIV VN assays. Currently, there are several different HIV neutralization assays formats under consideration and a lack of agreement on the most suitable assay. The CFAR is supporting a joint WHO/EU project (NeuNET) to evaluate and standardize HIV VN assays in an international collaborative study.

In the USA, a SARS-CoV repository has been established on behalf of the American Type Culture Collection (ATCC) in order to meet the needs of biodefence and the threat of emerging infections [82]. The type of reagents stored includes viruses, peptide arrays, monoclonal antibodies and proteins. It is hoped that an active collaboration can be established between NIAID and NIBSC in order to meet the expanding needs of the SARS research community.

Based on the discussion at the meeting, the following recommendations were made with respect to standardization of the immunological assays for SARS vaccine evaluation:

1. A WHO repository for SARS-CoV reagents ought to be developed. Collaboration between NIAID and NIBSC is recommended to achieve this goal.
2. Consensus must be reached for the reagents to be given priority in the repository.
3. An International Standard for SARS-CoV antibody is needed.
4. The most suitable source of antibody for the IS is convalescent human sera, but post-vaccination human sera could also be used.
5. A protocol for an international collaborative study aimed at validating the IS should be developed and distributed to prospective participants.
6. Collaborative study participants should be asked about their assay capabilities, e.g. number of sera, virus strains handled, etc. . .
7. The proposed IS collaborative study should include a core set of antibody preparations to be distributed and assayed in each laboratory (e.g. monoclonal antibodies, animal sera, other human sera).
8. Tests should be conducted using the same strain of SARS-CoV in each laboratory, but the different genetic lineages of SARS-CoV should also be represented in the study.

6. Biosafety issues

Biosafety issues associated with SARS-CoV vaccine development stem from the reports of laboratory-acquired infections in China. Sanofi Pasteur has adopted BSL 4 practices and BSL 3 equipment (e.g. Class 2 or 3 microbiological safety cabinets with respiratory protective equipment) for the preparation of SARS-CoV vaccines. Of note is the fact that SARS-CoV appears to be quite resistant to normal methods of virus decontamination (JF Saluzzo, personal communication). WHO has developed guidance, both general [83], and specific for handling SARS specimens [84].

7. Conclusions

The rapid success in the development of immunogenic and protective vaccines against SARS using a variety of platforms is encouraging, but should be tempered with concerns about the possibility of enhanced disease following exposure in vaccinated individuals [85]. Concerns mainly stem from reports of enhanced disease in FIPV-immunized or -infected kittens [63,66], from observations that antibodies elicited against certain coronaviruses mediate antibody-dependent enhancement of viral entry [65], and from the observation of inflammatory foci in liver tissue following SARS-CoV challenge in MVA-SARS-S vaccinated ferrets [38].

Candidate SARS vaccines will need to be evaluated in more than one animal model. They also will need to be thoroughly evaluated for the duration of the antibody response they induce, as well as for the breadth of their protective efficacy against different strains of SARS-CoV. The implications of the sequence heterogeneity among SARS-CoV strains are difficult to test at this time because the most divergent strains (civet SARS-like viruses) have not been recovered in culture.

Validation and international standardization of immunological assays for the evaluation of candidate SARS vaccines are essential to compare data across different trials. This requires the establishment of International Standards for SARS-CoV antibody and a repository for SARS-CoV reagents, with an international collaborative study to validate the ISs. The establishment of the repository by WHO in collaboration with NIBSC and NIAID was recommended.

List of meeting participants

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