Report of WHO Consultation on Needs and Opportunities for SARS Vaccine Research and Development

31 October-1 November 2003

Introduction and Meeting Objectives:

Joy Phumaphi, Assistant Director-General for Family and Community Health, welcomed the participants and stressed the importance of the consultation as part of WHO's overall efforts to prepare for a possible return of the Severe Acute Respiratory Syndrome (SARS). Dr Marie-Paule Kieny, Director of the WHO Initiative for Vaccine Research, offered introductory comments and summarized the meeting objectives and anticipated outcomes. She stressed the need for standardized protocols and reagents critical for vaccine development, for a better understanding of the SARS CoV antigenic and genetic diversity, and for further development of animal models that could facilitate SARS vaccine development and evaluation. Dr John Mackenzie summarized the recommendations made from two recent related WHO meetings that focused on research needs for SARS and on laboratory diagnostic issues. Dr Angela Merianos provided an overview of the global SARS outbreak and summarized the epidemiology and public health response that facilitated its control. Recommendations from a recent consultation on the clinical manifestations of SARS were not available at the time of this meeting, but were mentioned as relevant to these discussions, and WHO staff who had coordinated the clinical meeting were available throughout the discussions to assist as needed.

Summary of Presentations:

Clinical aspects and pathogenesis of SARS: Presentations from Canada and Hong Kong stressed that SARS occurred against a routine background of numerous patients suffering from atypical pneumonia, approximately half of whom never had a specific etiology attributed to their illness, and with many patients suffering from multiple concurrent infections. That situation represented a diagnostic challenge to the clinician and made it difficult to differentiate SARS from other causes of atypical pneumonia. In addition, the increased mortality rate for SARS seen among older age groups of patients is not unlike that seen for patients in this age group suffering from atypical pneumonia due to other causes. These observations support the need for a vaccine that will be effective in older populations, where it is well known that immune response to vaccination may not be as robust as that seen in younger age groups. A preliminary report on the analysis of chemokine and cytokine responses to SARS infection, as measured by gene expression profiles, suggested that elevated levels of certain cytokines may be indicative of the outcome of infection, and a better understanding of these responses may offer suggestions into treatment options as well a potential target for therapeutic intervention

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Coronavirus biology and genetic diversity: The availability of infectious clones of the SARS coronavirus (CoV) was discussed, as well as options that this may offer for future vaccine development efforts. New results were shared on sequence divergence of SARS CoV isolates recovered during the different outbreaks, and it became clear that virtually all cases detected in Canada, Vietnam, Taiwan and Singapore were either genetically similar or identical to those resulting from the super-spreader event documented at "Hotel M" in Hong Kong. Recent results of sequence analyses on isolates from patients from Hong Kong who were not associated with the Hotel M outbreak, as well as emerging data from China, suggest that other genetically diverse strains of SARS CoV exist and are capable of causing a SARS-like illness. Additional information on the genetic and antigenic differences among all isolates is urgently needed to ensure that vaccine candidates are effective in protecting against all SARS CoV strains. These observations can also provide additional molecular tools for epidemiological investigations, as was demonstrated during the consultation.

Animal models for SARS: A growing body of information suggests that non-human primates of various species are susceptible to infection with SARS CoV, but none tested to date appear to faithfully replicate the severe illness seen among human SARS patients. *Cynomolgus* monkeys experimentally infected with the SARS CoV develop a multifocal pneumonia, with evidence of infection noted by immunohistochemical analysis of macrophages and virus visualized by electron microscopy on samples taken from the throat and nasal passages. A specific immune response was detected in sera drawn from about day 10 post inoculation onward, and some monkeys developed a rash during acute infection. Similar results were seen when rhesus monkeys (*Macaca mulata*) were experimentally infected; no significant disease was seen, although monkeys did have mildly elevated temperatures and appeared sick. No evidence of viremia was seen, but 5 of 8 animals were positive for SARS CoV in lung and kidney tissues. Their immune response was characterized by low titers of neutralizing antibodies and evidence of a cellular immune response. Pathological examinations revealed typical interstitial pneumonia. No evidence of transmission of SARS CoV was seen from infected to non-infected monkeys housed in the same room. Younger animals (2.5 to 3.0 kg) appeared to be more susceptible to infection as compared to older, larger animals. Comments made during discussions suggested that African green monkeys (*Cercopethicus aethiops*) might also be susceptible to SARS CoV infection, however, no results were presented during the consultation to support this observation. Recent studies suggest that cats and ferrets are susceptible to SARS CoV infection and that ferrets develop a robust respiratory infection leading to disease. They stop eating soon after infection and suffer significant weight loss culminating in death around day 16 post-infection. Evidence of SARS CoV was found in several tissues and specimens taken from infected animals. While additional validation of this animal model is still required, it appears that the ferret may be valuable as a model for SARS CoV infection. The need for a standard challenge dose and consistent measurement of indicators of infection were discussed and agreed upon.

Experience with animal coronavirus vaccines: Several veterinary vaccines have been developed for use against coronavirus infections in domestic animals, and some of these

were discussed. Of greatest concern is the experience with inactivated vaccines against for feline infectious peritonitis virus (FIPV) infection, where administration of an inactivated vaccine sensitized cats to subsequent antibody dependent enhancement of infection, leading to more severe peritonitis and death. The spike protein of FIPV appeared to be responsible for enhanced infection of macrophages that resulted in rapid onset of acute peritonitis. The importance of determining if similar events occur with candidate SARS CoV vaccines in humans was underlined, and experiments to test this through passive antibody transfer and subsequent challenge in non-human primates were discussed. Information was also presented regarding vaccines to protect chickens against avian infectious bronchitis virus (IBV). Examples were cited where host genetic variation may lead to variable response to vaccination using an attenuated strain. Multiple serotypes of IBV are known to exist, and relatively poor heterologous protection between serotypes has been reported. These observations support the recommendation to obtain a comprehensive database to facilitate a better understanding of genetic and antigenic diversity among SARS CoV prior to final selection of candidate vaccines.

Strategies for SARS vaccine development: Several options exist for SARS CoV vaccine development and these were discussed during the consultation. Traditional inactivated vaccines are likely to be the easiest to produce both in terms of cost and time to produce, but the need to determine if antibody mediated enhancement occurs was stressed. Live attenuated vaccines may generate the best overall protection, but concerns about possible recombination or reversion to wild-type must be addressed. Subunit vaccines employing major gene products (such as spike protein) also hold promise as effective immunogens, with or without the addition of adjuvants. Experience gained in attempts to develop vaccines against HIV/AIDS or malaria have resulted in significant advances in the use of prime/boost strategies for immunization using DNA and vectored vaccines (vaccinia, adenovirus vectors for example), and these strategies hold promise for SARS vaccine development as well. The consensus opinion was that, to the largest extent possible, a variety of different candidate vaccines should be developed, at least through preclinical evaluation and comparison in animal models.

Progress in the development of candidate SARS vaccines: Both major vaccine manufacturers and smaller biotech firms have made significant progress in the initial development of candidate vaccines for SARS CoV. That was facilitated by the provision of funding by the U.S. National Institutes of Health to several companies to begin developmental efforts. Most manufacturers are using a SARS CoV seed virus isolate provided by the Centers for Disease Control and Prevention (CDC). This isolate (UTAH strain) was made from sputum from an acutely ill US traveler exposed in Hong Kong. The isolation was made under formal Good Laboratory Practices (GLP) using certified cells and media provided to CDC for this purpose by Aventis Pasteur. The isolate was fully sequenced and shown to be virtually identical to the Urbani strain of SARS CoV, and seed virus is available to manufacturers possessing appropriate containment facilities on request to CDC. Most manufacturers are growing the virus in certified Vero cells with serum-free media and report good growth to high titers. Many will start with the inactivated vaccine candidates, although alternate strategies including DNA, vectored vaccines, subunit products and live, attenuated candidates are also under consideration or

development. Vaccine manufacturers were encouraged to continue their developmental efforts using differing vaccine strategies, to ensure that the maximum number of options will be ultimately available. At least three different vaccine candidates (Sino-3, BJ-01 and GZ) are under development in China, and Phase 1 clinical trials may begin soon; other manufacturers anticipate clinical trials with inactivated vaccines to begin within 12-18 months.

Regulatory considerations: Regulatory officials from Canada, China and the USA attended the consultation and offered their suggestions as to how SARS vaccine development might progress most rapidly yet consistently with regulatory requirements for licensure. In general, all recommended that already approved cell lines and reagents, as well as validated and approved procedures and licensed facilities be used whenever possible to eliminate the need for costly and time-consuming validation of essential ingredients and processes. Manufacturers were encouraged to open and maintain an active dialogue with regulatory officials, starting early in the development process.

Recommendations

1. Key Research Needs:

It was recognized that a better understanding of the basic biology of the SARS CoV is required to underpin vaccine development, as well as other preventive and therapeutic strategies. The natural history of SARS CoV in humans and relevant animal models, as well as host-virus interactions *in vitro,* need to be better defined.

Key information gaps directly relevant to vaccine development need to be addressed, such as : (a) defining factors determining viral virulence; (b) correlates of host immunity; and (c) antigenic and genetic diversity of the virus.

Recommendation 1.1: The determinants of virus virulence, correlates of immunity, as well as viral antigenic and genetic diversity need to be defined. Standing working groups should be established for each focus area and group members should be encouraged to define research strategies, exchange information and discuss progress.

2. Reference Reagents and Standardized Testing:

Recommendation 2.1: Standard operating procedures and protocols should be developed for routine tests that will be required for vaccine development and analysis of host response to vaccination. These include neutralization tests, enzyme immunoassays, polymerase chain reactions (PCR), and others.

Recommendation 2.2: Well-validated reagents are needed as reference standards for essential laboratory tests, and these should be developed and made available to vaccine developers. Virus seed stocks, polyclonal and monoclonal antibodies,

recombinant proteins, PCR primer pairs and other reagents should also be made available if sufficient demand exists.

Recommendation 2.3: A common database should be established for genetic sequences and bioinformatics analysis of all known SARS CoV isolates, and this database should be linked to relevant clinical and epidemiological characteristics of each virus isolate.

3. Animal Models:

Animal models will be essential to the development and validation of candidate SARS CoV vaccines, and different species may provide different pieces of critical information. Consequently, efforts should continue to examine various species and strains of animals with the goal of developing models that will be useful for accurately determining the correlates of protection and to better understand potential vaccine-associated immunopathology. Mice offer the broadest array of well-validated host genetic diversity, and a systematic evaluation of various inbred mouse strains, including "knock-out" or "knock-in" genotypes, may significantly enhance understanding of SARS CoV pathogenesis and correlates of immunity. A recent report suggests that cats and ferrets are susceptible to SARS CoV infection and that they may faithfully replicate some or all of the clinical features of human SARS infection. Clearly, additional studies are needed to confirm and expand these observations. Finally, at least three species of non-human primates were reported to have been experimentally infected with SARS CoV. Each species appears to vary in susceptibility and response to infection, and further development of each potential model is required.

Recommendation 3.1: Efforts should continue to develop and validate animal models that faithfully replicate SARS CoV infection as seen in human, define correlates of human protect, and assist in determining if immune pathology results from SARS CoV vaccination.

Recommendation 3.2: A passive protection experiment in non-human primates should be done as soon as possible to determine if antibody-dependent enhancement of SARS CoV infection occurs.

A detailed discussion of relevant accomplishments in experimental animal model development was beyond the scope of this meeting, however, it was clear from the discussions that a significant amount of effort is underway in this area and that a dedicated meeting should be arranged within the next 3-4 months to bring together scientists working on animal model development to share information and discuss future efforts.

Recommendation 3.3: WHO should host a small meeting of scientists working on animal models for SARS within the next 3-4 months.

The need for standardized protocols to better define clinical or pathological end-points of infection was recognized. These should be developed and placed on a secure access website for discussion. Follow-up video conferencing might be appropriate to finalize these protocols.

Recommendation 3.4: Develop standard protocols for use in animal models, place on secure website for discussion, then finalize via videoconference or during meeting.

4. Production of Candidate Vaccines:

Various immunization strategies are under consideration to protect humans against SARS CoV infection. These include inactivated vaccines, subunit products, DNA either alone or in combination as part of a prime-boost strategy, vectored vaccines, and live, attenuated candidates. The field remains in its infancy and it would be premature to recommend one vaccination strategy over another. Similarly, alternative routes of vaccine administration (inoculation, aerosol, others) and formulation (adjuvants, others) should be considered.

Recommendation 4.1: Continue parallel development of various candidate vaccines and immunization strategies.

The most efficient path to development and licensure of a vaccine will involve utilization of existing, approved cells and substrates, licensed production facilities and validated procedures. These are well known to vaccine manufacturers and to regulatory officials. As development of candidate SARS CoV vaccines advances, manufacturers should maintain frequent contact with regulatory officials to ensure that all components and procedures to be used meet regulatory requirements and established standards. Manufacturers could present their vaccine development strategy and a summary of proposed processes to be used to regulatory officials at an early stage to ensure that potential regulatory hurdles are avoided.

Recommendation 4.2: Manufacturers should involve regulatory officials early and often during the course of vaccine development and manufacture.

5. Training and Capacity Building:

It was recognized that vaccine development for SARS CoV would require specialized training and equipment due to the hazardous nature of the virus. Biosafety guidelines should be developed that specifically address the safety and containment challenges to be faced by SARS vaccine developers, and training should be offered to those in need. In addition, there may be a need for additional BSL-3 containment facilities, especially areas where animals can be held under such containment, and guidelines for this may be required as well. One approach to meet this requirement might be through the establishment of international exchange programs for scientists likely to be involved in these efforts. Such exchange programs should not be limited to junior staff such as post-

doctoral fellows, but should include senior scientists and managers as well. Information exchange could be facilitated greatly through the use of focus groups and electronic media.

Recommendation 5.1: Prepare and distribute biosafety guidelines for use in the development and manufacture of SARS CoV vaccines and related activities including animal experimentation, and disseminate this information through focus groups, electronic media, and personnel exchanges and training fellowships.

6. Intellectual Property (IP) Considerations:

Given the successful worldwide collaboration initiated by the WHO on the identification and control of the SARS CoV, the SARS consultation group has addressed the possible impact of SARS CoV-related IP issues on the further progress of this process.

The SARS consultation group proposed that a strategy be developed, in consultation with stakeholders, to address potential SARS CoV-related IP issues and thus enhance development of intervention approaches. This strategy should aim to achieve consensus on SARS CoV IP issues for the benefit of public health.

7. Non-vaccine Interventions:

Although beyond the scope of this meeting, the participants nonetheless recognized the need for development of interventions beyond vaccines for use in the event of the return of SARS. Immune plasma or globulin, cocktails of humanized monoclonal antibodies, and antiviral drugs could all be extremely valuable in protecting individuals at high risk of infection or treating those infected. This will be especially true in the immediate future pending the availability of an effective vaccine. The group strongly encouraged development by industry of such products. The recommendations included in this report to establish standardized assays and reagents, and for further development of animal models for SARS CoV, will be equally valuable in the development of other therapeutic and preventive measures.

Recommendation 7.1: Development of immune globulin, other potential immunotherapeutics and antiviral drugs for the prevention and treatment of SARS CoV infection should be encouraged, in addition to vaccine development efforts.