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ABSTRACT

The world was struck by surprise when a Severe Acute Respiratory Syndrome (SARS) epidemic started in 2003 in China. This disease had never been observed in man before; the SARS-Coronavirus causing the disease was unknown. With the uncertainty about the future impact of this epidemic, an important international collaboration started spontaneously sharing scientific knowledge and reagents. Resources became quickly available, and public and private efforts were undertaken to develop rapidly a vaccine. We will discuss here the importance of the international collaboration and the availability of funding. Moreover, we will review the most important and challenging steps during the industrial development of the SARS vaccine highlighting the difficulties in terms of safety working
with such a highly pathogenic, unknown virus. We will emphasize the industrial perspectives on inactivation and decontamination experiments, the selection of the most promising vaccine candidate, the production process and the choice and use of animal models in such a pressing and difficult situation. Finally, we will briefly review the unique regulatory environment created during this period for the development of a SARS vaccine.

INTRODUCTION

In late 2002, several hundred cases of a severe atypical pneumonia were reported in the Guangdong Province of the People’s Republic of China. By the first quarter of 2003, similar cases were reported in Hong Kong and sporadically throughout South-East Asia and Canada. This severe disease, with a mortality of 5–10%, spread rapidly around the world and in April 2003, 25 countries on 5 different continents had reported cases [World Health Organization (WHO), accumulative SARS cases]. As a result, the WHO issued on March 2003 a global alert for the illness that would be known as “Severe Acute Respiratory Syndrome” (SARS) (WHO SARS alert). Within the same time frame, secondary cases of SARS were being identified in health care workers and family members who had close contact with patients suffering from this severe respiratory illness.

By the second week of June, using the WHO case definition (WHO case description), approximately 8000 SARS cases and 774 SARS-related deaths had been reported to the WHO. While the first wave of the SARS epidemic seemed to have reached its conclusion, it was completely unclear how the spread of the virus would evolve. There was no clear understanding of the animal reservoir and the impact of virus mutation and unapparent infections. Different situations could be envisaged; one scenario was that virulent SARS-coronavirus (SARS-CoV) would persist and become endemic. Another possibility was that other epidemic waves would occur or, finally, that the virus would disappear. Taking into account all the uncertainties and anticipating the worst-case scenario, many laboratories and vaccine manufacturers started working on a vaccine approach against SARS infection, largely based on what was known from animal CoVs.

In this chapter, we will discuss the necessity for international cooperation and the importance of discretionary funding for rapidly developing a prototype vaccine candidate. We will review the decision-making process, the strategic choices made in terms of vaccine candidate, adjuvants, working conditions, and the safety precautions implemented at the beginning and throughout the entire production process of the SARS vaccine. In addition, we will discuss the unique challenges associated with moving a vaccine such as SARS through the regulatory process.

ROLE OF INTERNATIONAL COLLABORATION AND U.S. NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES (NIAID)—LESSONS LEARNED

With the uncertainty about the possible extent of the spread of the SARS outbreak in early 2003, there was substantial international collaboration first in the isolation and identification of the CoV, sharing of sequence data, and eventually the sharing of seed virus that would be suitable for vaccine development. In the case of the sanofi pasteur vaccine, the prototype virus was supplied by the Centers for Disease Control and Prevention (CDC) (Utah Virus P2/Vero cell P143). Furthermore, in this spirit of collaboration, sanofi pasteur provided stocks of the Vero cells for the virus isolation to better ensure that any vaccine made from these cells would be acceptable from a regulatory perspective. These were the same Vero cells as are routinely used in the commercial production of inactivated polio vaccine. In addition, there was rapid sharing of immunological reagents to ensure that the plaque-purified prototype seed virus accurately reflected the circulating epidemic SARS-CoV.

One particular difficulty with rapidly emerging infections such as SARS, of course, is that they may occur anytime of the year and are unlikely to be aligned with the annual process for assigning financial and human resources and project prioritization. In order to circumvent these problems, once it became clear that the SARS-CoV could grow in Vero cells, the National Institute for Allergy and Infectious Diseases (NIAID) in the United States focused their request on several companies that had experience in large-scale Vero cell culture and had the experience to work with viruses at biosafety level 3 (BSL3) containment. Our first contact with NIAID on the possibility of developing an inactivated vaccine occurred in the first quarter of 2003. Sanofi pasteur was one of only two companies that satisfied the NIAID’s eligibility criteria. The solicitation and funding mechanism that NIAID employed was a directed Request for Proposals (RFP). This is sometimes called a Letter Contract by virtue of the fact that NIAID initiated contact with the companies first by letter outlining the project objectives, infrastructure requirements, and deliverables. The advantage of this mechanism, for diseases such as SARS, is that
the absolute eligibility criteria, vaccine characteristics, and project objectives are clearly defined; the proposal review cycle is very short; and the necessary financial resources are immediately available. In this particular case, the RFP was received by us on May 23, and we submitted our project plan and budget by June 16. Preliminary work began in July and August, and by the third week of September 2003, we had a fully executed contract. By October 2004, our inactivated prototype SARS vaccine was filled and available for clinical assessment. Another positive aspect of this mechanism is that involvement of such a large research organization provides the potential to access the expertise of many different investigators, as technical problems may arise. Unlike traditional research grants, these types of contracts have very short duration and, consistent with the sense of urgency, progress against objectives is monitored on a weekly basis, perhaps, contributing to the fact that our project finished almost a month ahead of schedule. Importantly, this type of mechanism does not compete with academic researchers for funding but allows these researchers to develop more basic science proposals of longer duration that can be reviewed and funded by other established mechanisms.

Perhaps, among others, there are three important lessons about responding to emerging threats that can be learned from the SARS experience. First, information must be shared quickly and transparently. The somewhat surprising observation that the SARS-CoV grew well in Vero cells was important in prioritizing the development of an inactivated, Vero cell–derived virus. Second, there should be an ongoing dialogue between the national research organizations and their potential industrial partners to understand what capacity and experience could be urgently brought to bear in a crisis situation. Although there are potential issues with competitive intelligence, it remains important that information about industrial capability should be shared. Third, funding organizations should have in place a mechanism for rapid proposal solicitation, review and monitoring, and also adequate discretionary funding that could support new vaccine projects in an urgent manner.

PRIOR TO THE START OF THE LABORATORY WORK

As early as April 25, 2003, the WHO and the CDC in the United States (WHO guidelines, CDC) recommended laboratories and vaccine manufacturers to handle SARS-CoV specimens using Standard BSL3 work practices. It is not exceptional for vaccine manufacturers to work in BSL3 facilities for the production of certain vaccines, e.g., rabies, Japanese encephalitis, or polio viruses. Some of these commercial vaccines have been made for decades. For these vaccines, robust production processes and standard operating procedures have been put in place. The experience gained on the decontamination and inactivation of viral vaccines during the production process and the quality control (QC) are all important. Moreover, the personnel working at the different stages of such a vaccine production are typically vaccinated, and revaccination procedures are in place in case of major accidents (i.e., boost after potential rabies contamination).

In case of an emerging virus such as SARS, the situation is completely different. Although the BSL3 experience will be fully exploited, all processes and procedures need to be discussed, evaluated, validated, and implemented. For each emerging virus, this exercise needs to be repeated and specific conditions must be adopted according to the unique characteristics of the new virus.

Consistent with the WHO and CDC recommendations, at sanofi pasteur live SARS-CoV was handled in a BSL3 facility. Since no treatment or vaccine was available against SARS, we decided to work in “BSL3 plus” laboratory conditions. Essentially our basic level of containment was BSL3 incorporating several BSL4 working practices: clothing change before entering the facilities, shower on exit, and all material decontaminated before exit from the facilities. Also, all steps, where the product was handled in open phase, were performed within Class III Biological Safety Cabinets (CSB). To prevent accidental contamination, the laboratory workers wore a positive pressure personal mask. These precautions were considered necessary taking into consideration the large amount of live virus handled (20–50 l) and finally turned out to be valid as the risk for contamination existed as was shown in the laboratory accidents in Singapore, Taiwan, and Beijing in China (Senior, 2003; Normile, 2004; Orellana, 2004). Furthermore, as described below, the decontamination experiments demonstrated that the SARS-CoV is an extremely resistant virus.

For the medical follow-up of the BSL3-trained personal involved in the SARS vaccine development, it was of critical importance to be able to distinguish the symptoms of respiratory distress caused by SARS or other respiratory agents. It was therefore decided that the BSL3 laboratory workers should be selected in accordance with their immune status and would be immunized with Streptococcus pneumoniae and influenza vaccines, if appropriate. In case a worker would present symptoms, a procedure had been put in place to isolate the worker using a high-efficiency particulate air (HEPA) filter mask before being transported to

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a nearby hospital where a special, dedicated negative pressure hospital room had been prepared.

**STARTING THE LABORATORY WORK: FIRST STEPS**

**Definition of the Vaccine Profile**

The very first decision concerned the choice of vaccine immunization strategy. In the intense early days of the epidemic a variety of approaches were considered. These included inactivated vaccines, subunit products, DNA (either alone or in combination as part of a prime-boost strategy), vectored vaccines, and live attenuated candidates. Similarly, alternative routes of vaccine administration (inoculation, aerosol, etc.) and formulation (adjuvants, etc.) were considered. In fact, several live attenuated and killed vaccines for veterinary CoVs are already marketed: e.g., a live attenuated several live attenuated and killed vaccines for veterinary CoVs are already marketed: e.g., a live attenuated

SARS Inactivation and Testing Reagents

When the laboratory work on the SARS-CoV vaccine development started, no data were available on the inactivation characteristics of the virus. One of the priorities was to identify the conditions to fully inactivate the virus for vaccine development, but also for decontamination of equipment, facilities, and waste decontamination. The results, as described in more detail below, were unexpected. The SARS-CoV is an extremely resistant virus and several of the routine decontamination working practices cannot be applied for this virus. These results demonstrate how important it is to immediately perform decontamination testing and adapt decontamination practices and strategies for each specific (emerging) virus.

At the time the development of a vaccine against an emerging virus is initiated, it is very unlikely that routine tests and reagents are available. This was indeed the case for SARS. Therefore, our first experiments were dedicated to develop routine tests that are required for vaccine development and the analysis of the host response after immunization. These include neutralization tests, enzyme-linked immunosorbent assays (ELISA), polymerase chain reactions (PCRs), and others. Well-validated reagents are needed as reference standards for essential laboratory tests as polyclonal and monoclonal antibodies, recombinant proteins, and PCR primer pairs. One of the most sensitive issues was how to select an appropriate animal model to evaluate the candidate vaccines. For example, we observed that NMRI mice gave a very heterogeneous response whereas Balb-C or C57BL/6J mice responded uniformly to immunization with the vaccine candidate. Also, guinea pigs appeared to be a good model for the evaluation of immune responses. Beyond immunogenicity, it is important to work with an animal model that is appropriate for challenge studies, as an assessment of vaccine efficacy. This is especially important for emerging infections since efficacy studies in humans may not be possible. In case of the SARS, the *Macaca fascicularis* was identified very early on (Fouchier et al., 2003) as a likely predictive, challenge model.

All of the work was done in constant communication with the regulatory authorities. Our experience with SARS reinforces the idea that manufacturers should be encouraged to open and maintain an active dialogue with regulatory officials, very early in the development process.

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THE PRODUCTION PROCESS

Viral Source and Raw Material

The SARS-CoV seed virus isolate was provided on August 25, 2003, by the CDC. This isolate (the so-called UTAH strain) was made from the sputum from an acutely ill U.S. traveler who had apparently been exposed in Hong Kong. This isolate was fully sequenced by the CDC and shown to be virtually identical to the Urbani strain of SARS-CoV.

To obtain an original seed virus, in full accordance with Food and Drug Administration (FDA) requirements, sanofi Pasteur provided certified Vero cells to the CDC, who performed the isolation of the virus and made two passages before sending the virus to sanofi Pasteur. Upon receipt, the virus underwent two additional passages, and was plaque purified. This plaque purification step is of importance to limit the risk of adventitious agents during the subsequent expansion of the virus. In contrast, there is also a risk that in selecting a plaque, it may differ significantly from the uncloned vaccine. To evaluate the latter, it was decided to compare cloned vs. uncloned virus in terms of virus sequence and immunogenicity in guinea pigs. It was demonstrated that the two candidate vaccines were totally similar.

Following this verification, the selected clone (VVNFL11) was passaged eight additional times for adaptation. From our experience with viruses to be used to prepare an inactivated viral vaccine at industrial scale, there is a need to reach a titer of virus >7.0 log/ml. Indeed, with the SARS-CoV, we obtained a consistent titer of around 7.3 log_{10} TCID_{50}/ml. In Vero cells, distinct cytopathic effect (CPE) is always observed at day 2 or 3 post-infection. These first
experiments encouraged us that the prototype vaccine could be produced in Vero cells using a single harvest totally compatible with our experience with inactivated poliovirus.

Raw materials used to develop the candidate vaccine (serum and trypsin) were selected in accordance with current regulation. Calf serum was imported from Australia, and gamma irradiated prior to use. The trypsin was from porcine origin and also gamma irradiated. Extensive evaluation for adventitious agents was performed on the raw material, which included the search for cytopathogenic agents, hemadsorbing agents, and specific viral contaminants as bluetongue, reovirus, rabies, parainfluenza type 3, specific bovine viruses [adenovirus, parvovirus, respiratory syncytial virus (RSV)], bovine viral diarrhea, rhinotracheitis virus), and porcine viruses (parvovirus, adenovirus; transmissible gastroenteritis virus; hepatitis E virus, rabies virus, and porcine pestis virus).

Manufacturing of the Viral Seed Lots

The viral seed lots were produced in Vero cells. QC testing is a major step in the qualification of such a seed. Of all the different tests performed (identification of the Vero cells, sterility, mycoplasma, titer, and contaminating viruses), the research of contaminating virus(es), also called adventitious agents, represents the most crucial step. To detect adventitious agents, sensitive cell culture monolayers of CV-1 cells, human diploid MRC-5 cells, and chick embryo fibroblasts (CEF) were inoculated with the crude viral suspension and are observed for induced CPE and/or hemadsorption. CV-1 cells are used in these tests, as they are of the same species and the same origin as the cells used for vaccine manufacturing. MRC-5 cells are human diploid cells and can potentially reveal other viruses able to infect human cells. And finally, taking into consideration the origin of the specimen (pulmonary syndrome) we added CEF, which are known to be sensitive to the infection of several respiratory viruses such as influenza virus and RSV. At the same time, adventitious agent testing was performed on control cells (search for hemagglutinating or hemadsorbent viruses) and on the supernatant of the control cells (search for adventitious agents) by inoculation of the three cell lines: Vero, MRC-5, and CEF. Adventitious agent testing was also done in vivo by inoculation in suckling mice, mice, and guinea pigs, as well as the allantoic cavity and yolk sac of embryonated chicken eggs.

Complementary to the conventional methods to qualify viral seed, as described above, extensive characterization was performed using PCR testing. The PCR tests are listed in Table 11.1. All tests were performed in accordance with international requirements, showing that no adventitious agents were detected.

Results of the Inactivation Experiments

The difficulty with inactivating viruses remains the balance between fully validated inactivation and preservation of immunogenicity or epitopes associated with protection. It is well known that reagents used to inactivate viruses [betapropiolactone (BPL), formaldehyde] can change the outer membrane antigens with the risk of a reduced immunogenicity of the vaccine. For the inactivation of SARS-CoV, we chose to test BPL. Assays were performed to determine the best BPL concentration for inactivation while maintaining a good immune response in mice. Virus

| TABLE 11.1 List of viruses for which the crude SARS-CoV viral suspension was monitored by PCR |
| Detection of viruses by PCR |
| Adenovirus |
| Bovine and porcine circovirus |
| Bovine herpesvirus—I, IV |
| Bovine polyoma virus |
| Cytomegalovirus |
| Epstein-Barr virus |
| Hepatitis A virus |
| Hepatitis B virus |
| Hepatitis C virus |
| Human herpes virus (HHV)-6 and HHV-7 |
| HHV-8 |
| Human immunodeficiency virus (HIV)-1 and HIV-2 |
| Human respiratory syncytial virus |
| Herpes simplex virus |
| Human T-cell leukemia virus type-1 (HTLV-1) |
| Human T-cell leukemia virus type-2 (HTLV-2) |
| Human papilloma virus |
| Human parainfluenza virus—1, 2, 3 |
| Human polyoma virus |
| Metapneumovirus |
| Parvovirus B19 |
| Simian spumavirus |
| Simian immunodeficiency virus |
| Simian retrovirus—1, 2, 3/SMRV |
| Simian T-cell lymphotropic virus |
| Simian virus 40 |
inactivation was performed using three different BPL concentrations: 1/2000, 1/3000, and 1/4000 (v/v). In our experience, a 1/4000 dilution of BPL was the optimal concentration for the inactivation of SARS-CoV based on a balance between total inactivation and maintenance of antigenic properties. The 1/4000 BPL dilution is similar to what is used for rabies vaccine inactivation.

The usual way to measure viral inactivation is by kinetic studies, i.e., reduction in virus infectivity. This technique has a detection limit of 1.5 log TCID50/ml. The kinetics of inactivation was performed at 0, 5, 30 min, and 1, 2, 3, 4, 6, 8, and 24 h, and it was shown that inactivation below the limit of detection was obtained after 6 h. In order to increase the detection sensitivity, an amplification test was also performed. For this amplification, Vero cells are incubated with a portion of the viral solution following inactivation for different periods of time. After 7 days of incubation, the cells are trypsinized and cultivated for additional 14 days. At the different stages of amplification, the cells were microscopically observed for CPE and at the end of the incubation (day 21) an immunocolorimetric assay test was performed. Our data demonstrated that the virus is fully inactivated by 12 h, not 6 h of BPL treatment, demonstrating that the amplification test is much more sensitive.

To complete the validation of the amplification test, the minimum limit of detectable infectious viral particles was determined. This was done by spiking the inactivated vaccine with different concentrations of live virus and incubating with Vero cells. Using this approach, it was possible to establish the minimum virus detection as 1 pfu.

Based on these data, it was concluded that inactivation with 1/4000 BPL dilution for 12 h fully inactivates the SARS-CoV batches. To ensure a very large safety margin, we adopted an inactivation period of 24 h for the SARS-CoV vaccine production process.

**ANIMAL MODELS**

Since a direct efficacy trial in humans will be impossible, because of a lack of naturally circulating SARS-CoV, the licensure of a SARS-CoV vaccine will depend on surrogate markers. Recently (as described below) the FDA adopted the Animal Efficacy Rule that envisions that under such circumstances, demonstration of efficacy can be performed in two animal models. For SARS-CoV vaccine development, monkeys and ferrets can be used to evaluate candidate vaccine. Both animal models show pathology in the lungs upon autopsy.

The immunogenicity of the SARS vaccine was evaluated in nonhuman primates, M. fascicularis, and ferrets. Both animal models are susceptible to infection, do show some signs of disease (lethargy), and show signs of pulmonary lesions upon histological examination (Fouchier et al., 2003; Martina et al., 2003; ter Meulen et al., 2004; Rowe et al., 2004; McAuliffe et al., 2004). Different doses of the SARS vaccine (6 or 7 log10 TCID50/ml) were injected in the presence or absence of aluminum hydroxide. Two intramuscular injections were performed at a one month interval.

Regarding the humoral response, sustained levels of ELISA and serum-neutralizing virus-specific antibodies were elicited in vaccinated monkeys and ferrets. A significant dose–effect relationship could be demonstrated. Moreover, a strong adjuvant effect of aluminum hydroxide was evidenced for each vaccine dose and proved in most cases to be highly significant.

In order to evaluate the efficacy of the SARS vaccine, immunized monkeys and ferrets were challenged intratracheally with a heterologous Hong Kong SARS-CoV strain (Coronovative, Rotterdam, The Netherlands). Monkeys immunized with 6 or 7 log10 of inactivated virus were protected as measured by RT-PCR and viral titration on lung samples five days post-challenge. The ferrets were protected at the lower immunization dose of 5 or 6 log10.

Based on our experience to date, the inactivated, adjuvanted SARS-CoV prototype vaccine seems to be a good candidate for further evaluation in Phase 1 studies.

**REGULATORY APPROVAL PROCESS—SOME UNIQUE ASPECTS OF SARS AND OTHER EMERGING DISEASES**

As with other vaccines, vaccines for SARS and other emerging threats need to follow a structured pattern of regulatory development. The initial stages would be very similar to those followed for vaccines under development for conventional infectious diseases. In the United States, the earliest stages would include the development of sufficient preclinical information about the vaccine to allow the preparation of an investigational new drug (IND) application for submission to the FDA (see Chapter 13). The IND may have information unique to the vaccine candidate but should include information about the rationale for the vaccine design, the source of the virus and other components, the manufacture of the active vaccine component, formulation, preliminary characterization of the vaccine

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including purity and potential contaminants, immunological testing, and animal testing, including toxicology. Even at this early stage, the vaccine should be made under GMP conditions and other laboratory work conducted under good laboratory practice (GLP) conditions, as appropriate. The IND application should also include important information about the Phase 1 clinical design, focusing on how the safety will be monitored and a discussion of any potential adverse reactions based on the experience with vaccines that have similar components or methods of preparation. There should be an opportunity to outline the vaccine concept and Phase 1 clinical study at a pre-IND meeting that often provides the opportunity to receive the input and concerns of the regulatory agency.

Following approval of the IND, vaccines such as SARS can progress to a conventionally designed Phase 1 study. Typically, this Phase 1 clinical study is descriptive and would include a small number of healthy young adults with the emphasis on monitoring the safety of the vaccine (local and systemic reactions). Often, the first immunologic assessment is part of this study. Following successful completion of the Phase 1, as with other vaccines, a SARS vaccine candidate could move forward to Phase 2. During this phase, in addition to safety monitoring, dose-ranging studies are conducted in much larger groups of individuals and the vaccine should meet predefined primary and secondary endpoints. If the Phase 2 is successful, following a pre-Phase 3 meeting, clinical studies are conducted in a greater number of subjects during which less frequent reactions can be detected and the efficacy or effectiveness of the vaccine determined. As part of this Phase 3 evaluation, the consistency of sequential lots of the vaccine are typically compared in order to ensure that the vaccine can be reproducibly manufactured.

Obviously, as the vaccine progresses clinically from Phase 1 to Phase 3, the size of the lots of vaccine often increases and the manufacturing and in-process and release testing specifications become increasingly well defined, so as to guarantee that the vaccine can consistently be made at a commercially useful scale. If all three phases of clinical development are successful, the manufacturer may then submit a Biologics License Application (BLA), which is a very extensive compilation of all of the information relating to the development and manufacture of the vaccine.

As suggested in the Animal Models section above, the unique challenge for SARS and other emerging threats, whether anthrax or Ebola viruses, is that it may not be possible to conduct Phase 3 clinical studies to determine the effectiveness of the vaccine. For these agents, it would be too dangerous to conduct challenge studies in humans and the prevalence of the disease is either nonexistent, sporadic, or too small to allow the development of a reasonable clinical protocol. In anticipation of this problem, largely in the face of potential bioterrorist agents, in 2002, the FDA adapted the so-called Animal Rule [see Federal Register, May 31, 2002 (Volume 67, Number 105)]. Under this guidance, new drugs or biological products that are intended to prevent serious or life-threatening conditions may be approved on evidence of effectiveness derived from appropriate studies in animals and any additional supporting data, if controlled clinical studies cannot be conducted in human volunteers and field trials are not possible. In order to satisfy this alternative mechanism, however, several criteria must be met. First, there is a reasonably well-understood pathophysiological mechanism that can be ameliorated or prevented by the product. Second, the effect is demonstrated in more than one animal species, unless it is demonstrated in a single species that represents a sufficiently well-characterized animal model. Third, the animal study endpoint is clearly related to the desired benefit in humans. And finally, the data are sufficiently well understood to allow selection of an effective dose in humans. It is therefore reasonable to expect that the effectiveness of the product in animal model(s) is a reliable indicator of its effectiveness in humans.

Obviously, it is too early to know whether the SARS vaccine candidate as described in this chapter will move forward and be able to meet all of the criteria of the Animal Rule. In particular, SARS vaccine development is hindered by relatively little information about human CoVs in general. Until the rapid emergence of SARS, most of the basic research was focused on animal CoVs and our inactivated SARS vaccine candidate described in this chapter is exclusively based on experience with vaccines to animal CoVs. Certainly, it is too early to conclude whether the ferret and/or M. fascicularis is/are the most appropriate model(s) for human SARS infections. As a result, except for clinical cases documented during the outbreak, there is relatively little information about SARS pathogenesis and correlates of immunity. Another difficult aspect is that a feline infectious peritonitis (FIP) vaccine was actually harmful to the health of the immunized cats upon challenge with wild-type FIP virus (Weiss and Scott, 1981). Before moving forward with approval, therefore, it will be very important to determine whether these adverse outcomes can be prompted or mimicked by any of the SARS vaccine candidates.

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CONCLUSIONS

What Were the Parameters for the Rapid Development of the SARS Vaccine?

The production of a GMP clinical lot of a monovalent, whole, inactivated, aluminum hydroxide-adjuvanted SARS-CoV vaccine took 12 months. In terms of vaccine development, this is extremely rapid. Several factors contributed to these short timelines.

The grants made available by the NIAID for the development of a SARS vaccine completely changed the classical environment, allowing vaccine industries to start almost immediately the development of a new vaccine. Indeed, the development of a new vaccine can only be done to the detriment of other vaccine developments, mobilizing teams and facilities.

From a technical point of view, the choice of a classical vaccine development strategy using conventional procedures, such as Vero cell culture for viral propagation and BPL inactivation, was a decisive factor to success. Importantly, we were able to quickly recruit a volunteer workforce that was both familiar with the technology and trained to work in a BSL3-plus environment.

A close collaboration with the reference laboratory, the CDC’s Influenza Branch, where the SARS-CoV was isolated, was essential. We provided certified Vero cells to the CDC, which allowed us, upon receipt of the purified strain from the CDC, to re-isolate the SARS-CoV under conditions making the prompt start of a vaccine development possible.

When initiating vaccine development against a new emerging infectious agent, the problem of availability of reagents and routine tests to perform biological and molecular studies must be addressed. It is obvious, that at the beginning of such development, there are no such reagents or commercial kits available. As a consequence, the first step in the SARS-CoV project was to prepare the different reagents (antisera and monoclonal antibodies) and the appropriate tests (viral titration, PCR, ELISA, immunofluorescence assay, etc.). Finally, a series of preliminary experiments on monkeys (three months after the start of the project) had given guidance whether it was appropriate to use an inactivated vaccine, as well as to the choice of the adjuvant.

Constant communication with regulatory authorities has allowed the validation of this strategy from the beginning of the project. This communication was also very important for the qualification of the viral seed lots. The qualification of the Vero cells was not an issue as several vaccines are already produced in Vero cells, but this was obviously not the case for the viral seeds. Two major obstacles had to be overcome: (1) the realization that the animal testing had to be done in BSL3 facilities by BSL3-trained personnel, and (2) the search for adventitious agents using general classical tests (search for adventitious viruses on cells and in animals) and specific tests (PCR). For the latter, there was no list available and the final testing to be performed was under the responsibility of health authorities. This resulted in a rather exhaustive list of PCR testing.

Can We Shorten the Timelines Even More When Facing an Emerging Pathogen?

It is likely that epidemics will emerge in the future from unrecognized sources and some of these will be highly pathogenic for humans. These pathogens will be categorized as BSL3 or BSL4 pathogens needing high security level laboratories as well as specialized personnel. How to manipulate these pathogens that are highly pathogenic, in large quantities? To face the emergence of new pathogens, dedicated structures are needed with the right equipment and trained personnel. From an industrial perspective, this seems not compatible with the need and use of trained personnel and facilities that do have a constant activity to assure the production of existing vaccines and the development of new vaccines. Such emergency structures could be set up and maintained by national reference centers respecting the BSL requirements as well as the GMP conditions. It would be very beneficial for industries to collaborate with such reference centers that provide purified pathogens and reagents allowing a prompt start of a vaccine development.

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