Chapter 12

Severe Acute Respiratory Syndrome

Early in 2003, an outbreak of the until then unknown severe acute respiratory syndrome (SARS) was reported in southeastern People's Republic of China. The outbreak was thought to have first emerged in the Guangdong province in November 2002. Subsequently, the infections spread to Hong Kong (February 2003) and other countries of Southeast Asia, including Vietnam, Taiwan, and Singapore, as well as to Canada and the United States (http://www.fda.gov/oc/opacom/hottopics/sars/).

Epidemiologic studies have shown that the disease disproportionately affected health care workers (21% of all cases) and close contacts of SARS patients, such as family members. No new cases of SARS have been reported since April, 2004 (http://www.who.int/scr/don/2004_04_30/en). There has been some evidence from research to suggest the presence of a variation in an immune system gene that may make people with the variation much more vulnerable to the SARS-associated coronavirus (SARS-CoV). This genetic variation is more common among people of Southeast Asian descent but is rare in other populations. This may help explain why most SARS cases have occurred in China and Southeast Asia.

According to statistics by WHO, a total of 8,098 people worldwide contracted SARS during the 2003 outbreak. Of these, 774 died (overall fatality rate: 9.6%). In the United States, only 8 people had laboratory evidence of SARS—all of them had traveled to other parts of the world where SARS was present. Higher mortality has been observed in older patients and in patients with comorbid conditions, such as diabetes, lymphopenia, and liver dysfunction (http://www.cdc.gov/ncidod/sars).

The novel SARS-CoV was identified as the cause of SARS. Previously identified human coronaviruses (named for their spiky, crown-like appearance) are known to cause only mild respiratory infections (e.g., common cold). It now seems likely that SARS-CoV had evolved from one or more animal viruses into a completely new strain.

The SARS-CoV spreads primarily through close human contact (e.g., droplets, airborne particles in face-to-face contacts). Touching a SARS-CoV–infected surface and subsequently touching the eyes, nose, or mouth may also lead to infection. SARS typically begins with flu-like symptoms, including high fever (100.4°F/38°C, or higher) that may be accompanied by headache and muscle aches, cough, and shortness of breath. In some cases, the fever may not appear for up to 10 days. Up to 20% of people infected may develop diarrhea. Most patients with SARS subsequently will develop pneumonia. Between 10% and 20% of SARS patients will become progressively worse and develop breathing problems so severe that they may require the help of a mechanical respirator. SARS is fatal in some cases, often due to respiratory failure. Other possible complications include heart and liver failure.

12.1 NIAID Agenda for SARS Research

In spite of a concerted global effort, there is still no effective treatment for SARS. A combination of antiviral drugs normally used to treat AIDS—lopinavir-ritonavir along with ribavirin—have been shown in clinical studies to prevent serious complications and deaths from SARS. However, further studies are needed. In August 2004, the SARS-CoV was added to NIAID's List of Category C Priority Pathogens for Biodefense (http:// www2.niaid.nih.gov/biodefense/bandc_priority.htm). Funding opportunities are listed under NIAID's biodefense programs (http://www2.niaid.nih.gov/biodefense/research/funding.htm).

On May 30, 2003, NIAID convened an international meeting on SARS to develop a robust research agenda focused on the discovery of effective therapies to control the disease.

A number of research goals and objectives have been recommended:

- Expand research efforts on the basic biology of the virus, including studies on replication, biodiversity, factors that influence transmission to humans, and the development of animal models

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- Determine the basis of SARS-associated immunopathology and identify the components of innate and protective immunity, and the impact of polymorphisms on the disease outcome
- Support the rapid development of multiple vaccine strategies
- Expand capacity for *in vitro* evaluation of antiviral drugs with activity against SARS, and identify viral and host targets for therapeutic intervention
- Develop diagnostics that are rapid, sensitive, and easy to use and that can be widely distributed
- Define SARS disease progression, persistence, correlates of immunity and susceptibility, or resistance to reinfection
- Expand surveillance to identify the animal reservoir(s) and factors that influence the spread of the virus, and assess whether immunocompromised individuals, children, and pregnant women are at an increased risk
- Provide the research community with resources, including opportunities to upgrade biocontainment facilities, and provide standardized reference reagents, including microarrays and tetramers

12.1.1 Research Programmatic Developments

In response to the need for a rapid increase in research on the SARS coronavirus, NIAID has initiated a vigorous program to support the development of diagnostics, vaccines, and therapeutics for SARS (http://www.google.com/search?hl=en&q=niaid+infergen+sars). Several major programs of NIAID-supported SARS research include:

- A clinical protocol to evaluate interferon-alfacon-1 (Infergen, Amgen, Inc.), an engineered recombinant interferon molecule that has a potent anti-SARS-CoV activity in an *in vitro* assay for cytopathic activity. Infergen has previously been known for its anti-hepatitis C activity.
- NIAID's Vaccine Research Center (VRC) has contracted with Vical, Inc., to manufacture a single-dosed, circular DNA plasmid-based vaccine encoding the S protein of the SARS-CoV. *In vitro* studies have demonstrated that this vaccine induces T-cell and neutralizing antibody responses, as well as protective immunity. A Phase I open-label clinical study to evaluate safety, tolerability, and immune responses was completed in May 2006. In the study, healthy subjects were administered 4.0 mg DNA vaccine doses at three 1-month intervals. The vaccine was well tolerated.
- Several animal models have been developed for SARS, including mouse, hamster, and non-human primates. None of the animals tested exhibited clinical disease after intranasal administration of the virus but rather exhibited antibodies against SARS-CoV and cleared the virus.
- NIAID Biodefense Proteomics Research Program Contract "Identifying Targets for Therapeutic Interventions Using Proteomic Technology" has been implemented, and seven centers have been funded (http://www.niaid.nih.gov/dmid/genomes/prc/default.htm).
- A NIAID grant supplement to China's CDC and its collaborators has initiated the development of three different SARS projects: (i) development of immune correlates of protection through the study of pediatric and adult serum, stool, and cellular clinical samples obtained longitudinally from SARS patients; (ii) development of a panel of human SARS-CoV antisera that can be used to standardize diagnostic assays (in collaboration with CDC and FDA); and (iii) identification of animal reservoirs of SARS-CoV.

12.2 Recent Scientific Advances

- *Identification of SARS-CoV ORF Structures.* To date, the three-dimensional structures of seven open reading frames (ORF) structures have been elucidated, namely (i) ORF 1a/nsp3b (phosphatase); (ii) ORF 1a/nsp5 (3CL-pro); (iii) ORF 1a/nsp7 (with four-helix bundle); (iv) ORF 1a/nsp9 (RNA binding domain); (v) ORF1a/sars7a (unknown function); (vi) ORF 1a/nsp10 (contains zinc finger); and (vii) ORF 1a/nsp3d (PLpro-protease with deubiquitinating activity). Their determinations have been accomplished after cloning, expression, and x-ray crystallography (http://www.proteomicsresource.org/Meeting/May2007/presentations/2007_May_PRC2007_FSPS_SSS.pdf).
- *Inhibition, Escape, and Attenuated Growth of SARS-CoV Demonstrated with Antisense Oligomers.* Peptide-conjugated antisense morpholino oligomers (P-PMOs) were designed to bind by base pairing to specific sequences in the SARS-CoV (Tor2 strain) genome *(1)*. The P-PMOs were tested for their capacity to inhibit the production of infectious virus, as well as to probe the function of conserved viral RNA motifs and secondary structures. The P-PMOs tested were found effective when administered at any time prior to peak viral synthesis and exerted sustained antiviral effects. After several viral passages in the presence of regulatory sequence-targeted P-PMO, partially drug-resistant SARS-CoV mutants arose that grew more slowly than did wild-type SARS-CoV. These results suggested that the P-PMO compounds tested exhibited a powerful therapeutic potential against SARS-CoV.
- *Supramolecular Architecture of Severe Acute Respiratory Syndrome Coronavirus Revealed by Electron*

Cryomicroscopy. In a recent report *(2)*, the two-dimensional images of the S, M, and N proteins of SARS-CoV and two other coronaviruses at a resolution of approximately 4 nm have been described. Trimeric glycoprotein S proteins were in register with four underlying ribonuclear densities. The ribonuclear particles displayed coiled shapes when released from the viral membrane. This is the first detailed view of coronavirus ultrastructure and will help in understanding the coronavirus assembly pathway.

- *The SARS-CoV Cysteine Protease, the Papain-like Protease (PLpro), Is Identified and Structurally Characterized.* The replication of SARS-CoV and other coronaviruses is dependent on processing of replicase polyproteins by two cysteine proteases, one of which is the papain-like protease (PLpro). By using bioinformatics analyses of multiple SARS-CoVs, researchers have been able to identify a putative catalytic triad and a zinc-binding site *(3, 4)*. Furthermore, molecular modeling of PLpro suggested deubiquitinating activity. The 1.85 Å crystal structure of the PLpro catalytic core was then elucidated, demonstrating an intact zinc-binding motif, a catalytically competent active site that includes a ubiquitin-like amino terminal domain, as well as overall resemblance to known deubiquitinating enzymes. Sites within the catalytic cleft were well defined and accounted for strict substrate-recognition motifs. The detailed understanding of the SARS-CoV Plpro enzymatically active domain is critical to the development of antiviral drugs and to better understanding of the role of PLpro in the biogenesis of the SARS-CoV replicase complex.
- *Human Antibodies That Block Human and Animal SARS Viruses Identified.* An international team of investigators has identified the first human antibodies that can neutralize different strains of virus responsible for outbreaks of SARS *(5)*. The researchers used a mouse model and *in vitro* assays to test the neutralizing activity of the antibodies. The study is important because the viral strain that caused the outbreak in people in 2002 probably no longer exists in nature, and what is needed is a proof that the antibodies are effective not only against the strain of SARS virus isolated from people but also against a variety of animal strains, because animals will be a likely source for re-emergence of the SARS virus. The investigators' research into the spike glycoprotein—the part of the virus that binds and allows entry into human cells—provided the knowledge needed to identify several human antibodies against the SARS virus. In particular, the researchers identified two human antibodies that bind to a region on the SARS virus' spike glycoprotein, which is called the receptor-binding domain (RBD). One of the antibodies, called S230.15, was found in the blood of a patient who had been infected with SARS and later recovered. The second antibody, m396, was taken from a library of human antibodies the researchers developed from the blood of 10 healthy volunteers. Because humans already have immune cells that express antibodies that are very close to those that can effectively neutralize the SARS virus, m396 could be fished out of healthy volunteers. The investigators next solved the structure of m396 and its complex with the SARS RBD and showed that the antibody binds to the region on the RBD that allows the virus to attach to host cells. If the antibodies were successful in binding to the SARS RBD, they would prevent the virus from attaching to the SARS coronavirus receptor, ACE2, on the outside of human cells, effectively neutralizing it. When tested in cells in the laboratory, both antibodies potently neutralized samples of the virus from both outbreaks. The antibodies also neutralized samples of the virus taken from wild civets (a cat-like mammal in which strains of the virus were found during the outbreaks), although with somewhat lower potency. The discovery of two effective antibodies has the advantage that a newly emergent variation of the SARS coronavirus might be insensitive to neutralization with one, but still susceptible to the other. The results of the study have demonstrated novel, potential antibody-based therapeutics against SARS that could be used alone or in combination *(5)*.

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