華夏英才基金學術文庫

Reiner. Zhao. Chen. Guo.

生物技术与药物学前沿

Frontiers of Biotechnology & Pharmaceuticals

第四卷

Volume Four

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Preface to Volume IV in the Frontiers of Biotechnology and Pharmaceuticals Series.

In early 2003, a new human virus named SARS (for sudden acute respiratory syndrome) began terrorizing Asia, and due to its particularly infectious nature, health officials had their hands full trying to contain the outbreak. An important aspect in combating SARS was the development of a quick and reliable diagnostic test to determine if a patient was infected with the SARS virus. An overview of the SARS epidemic and the development of a DNA based bead array analysis to diagnose the SARS viral infection begin a special section on antiviral research. Also included in this section are reviews on several major viruses that continue to plague humans. A new way of combating HIV is described which utilizes CCR5 antagonists to block the first step in the viral infection process, entry into the host cell. Another virus of growing concern is hepatitus. Analogous to treatments for HIV, nucleosides and their analogs are proving to be effect against hepatitus B. Therapeutic agents which target for inhibition a key enzyme in the viral replication cycle, HCV protease, are being investigated. Although not with the lethality of HIV or hepatitus, herpes and the cold virus infect enormous numbers of people. Chapters on the status of both rhinovirus protease inhibitors and treatments for herpes conclude this section.

The second section of the volume consists of a variety of biotechnology and drug discovery topics. Using DNA microarray technology, gene expression analysis is being used to identify the causes of brain aging and neuro-degenerative diseases such as Alzheimer's disease. The SUMO protein and its ability to regulate other proteins and their pathways, which may be relevant in a number of diseases and drug targets, is reviewed. A perhaps overlooked tool for proteomics research, the immunoglobin-Y antibodies isolated from egg yolk, is described. The alpha-helix is one of the key structural features in proteins, and the ability to mimic this motif in a small molecule could have important applications in drug discovery. The state of the art in the design and synthesis of small molecule alpha-helix mimetics and their application to the area of modulating protein-protein interactions are summarized. The improved design of inhibitors of protein kinases has been accelerated by being able to identifying specific amino acid residues in the

kinase active site area that can be exploited for maximal differentiation (inhibitor selectivity). Reviews on RNA as a drug target, including examples from antibacterial and antiviral research, the use of NMR as a tool for guiding drug discovery, and a compilation of new drugs approved by the FDA over the past year complete this section.

This volume encompasses a wide variety of research topics that are pushing the frontiers of drug discovery and the pharmaceutical sciences, and are continually redefining the current and future states of human health. Although, as is evident from the significant progress made in the past few years, physicians have more choices in the treatment of diseases than ever before, yet, there remain many unsolved problems. As with SARS, there will be other new diseases for which people will be susceptible, and the necessity of further discoveries will certainly not diminish.

I. Antiviral Research

II. Biotechnology and Drug Discovery

SARS OUTBREAK AND THE DIAGNOSTIC TECHNOLOGY

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Abstract

The Severe Acute Respiratory Syndrome (SARS) outbreak was recognized as a global threat in March 2003, and was completely contained by June. Knowledge about the epidemiology and ecology of SARS coronavirus infection remains limited. Non-specific clinical features of SARS indicate an urgent need for rapid differential testing for early alert and better management of the epidemics. The differential test reported by Han in this volume stands at the frontier of diagnostic technology. It detects 10 microorganisms involved in acute respiratory infections: SARS virus, Respiratory syncytial virus, arainfluenzaviruses type 1 and type 3, Influenza A and B viruses, Enterovirus, Adenovirus, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*. The multiplex reverse transcription-PCR (RT-PCR) assay amplifies and detects all potential pathogens together in 3 hours, and is capable of testing hundreds of samples per day with the Luminex xMAP technology platform.

Early detection is the priority in the control and management of the SARS epidemic. According to a News Release by the CDC AIDS Center in China, the first RNA diagnostic test for SARS was approved by the SFDA on October 14, 2003. It was reported that this test detected 46.4% (13/28) of stool samples and 47.4% (28/59) of plasma samples from the 0-5 day onset patients. Over 50% of the samples from the 6-10 day onset patients were detected positive. This method was observed at Shenzhen PG in excellent consistency of 97.9% (47/48) in a comparison between PG and the first approved SARS diagnostic test, German ARTUS, estimating PG = 0.93(ARTUS) + 1.79 (n = 48).

This chapter cites Singapore's SARS outbreak in September, evidently linked to a lab contamination. Other RT-PCR tests are mentioned along with WHO and CDC SARS clinical and case definitions, and some guidelines for SARS diagnosis for background information. In responding to some key issues

and questions about SARS etiology and diagnosis, the Fact Sheet for Clinicians by CDC, and the WHO news reports and consensus are quoted, since they have provided the most valuable and thorough information up to date.

SARS outbreak in 2003, the knowledge is limited

The Severe Acute Respiratory Syndrome (SARS) outbreak, first recognized as a global threat in mid-March 2003, circled around the world in a chain reaction, and was completely contained in less than four months. On July 5, 2003, the World Health Organization (WHO) reported that the last human chain of transmission of SARS had been broken.(1) Though WHO has declared the SARS epidemics over, it is, so far, the biggest event of public health in 2003, and has brought disastrous consequences to social life and the global economy. Much has been learned about this syndrome since its outbreak in March 2003, including its causation by a new coronavirus (SARS-CoV), and the genomic sequence of the new virus; however, our knowledge about the epidemiology and ecology of SARS coronavirus infection and of this disease remains limited. (1)

Non-specific clinical presentations of SARS patients demand a rapid differential test

SARS is a disease caused by SARS coronavirus (SARS-CoV). Nosocomial transmission of SARS-CoV has been a striking feature of the SARS outbreak. The majority of the cases are adults. The mean incubation period is 5 days with a range of 2-10 days although there are isolated reports of longer incubation periods. There have been no reports of transmission occurring before the onset of symptoms. The Natural history of the disease showed that in the first week of illness, it appeared that patients initially develop influenza-like prodromal symptoms. Presenting symptoms include fever, malaise, myalgia, headache, and rigors. No individual symptom or cluster of symptoms has proven specific. Although history of fever is the most frequently reported symptom, it may be absent on initial measurement. (2,3)

The WHO case definitions during the outbreak period relied heavily on epidemiological criteria to increase the specificity of syndromic clinical criteria

for atypical pneumonia or respiratory distress syndrome (RDS). However, epidemiological links to cases of SARS and areas reporting recent local transmission are no longer of use in helping to define incident cases. Furthermore, the difficulties of controlling the epidemics is due to the non-specific clinical features of SARS and the lack of a rapid diagnostic test that can reliably detect SARS-CoV in the first few days of illness. The seasonal occurrence of other respiratory diseases, including influenza, may confound any surveillance for SARS or demand a level of quality and intensity which few health care systems worldwide can sustain. Even with the most sophisticated surveillance systems, the first cases of SARS in the post-outbreak period may escape early detection. (4)

It is almost impossible to definitively distinguish between influenza and SARS based on clinical symptoms in its early stage. Antibody ELISA tests alone cannot serve as a final diagnostic tool. CDC requires that initial diagnostic testing for suspected SARS patients should include a chest radiograph, pulse oximetry, blood cultures, sputum Gram's stain and culture, and testing for viral respiratory pathogens, notably influenza A and B and respiratory syncytial virus. A specimen for Legionella and pneumococcal urinary antigen testing should also be considered. Clinicians should save any available clinical specimens (respiratory, blood, and serum) for additional testing until a specific diagnosis is made. (5,6)

Significance of differential testing and current status of RT-PCR

Rapid differential diagnosis is important for a timely response to the SARS outbreak. We are delighted to have Jian Han's chapter "SARS Differential Diagnosis with a Bead Array Method" in Volume 4 of the book series "Frontiers of Biotechnology and pharmaceuticals" in 2003. In Han's chapter, he reports a molecular diagnostic method that detects 10 microorganisms causing acute respiratory infections which in addition to SARS virus include: Respiratory syncytial virus, Parainfluenzaviruses type 1 and type 3, Influenza A and B viruses, Enterovirus, Adenovirus, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*. This multiplex reverse transcription-PCR (RT-PCR) assay amplifies all potential pathogens in one tube. The RT-PCR test is a requirement of CDC for detection of SARS-CoV RNA.(7) The

pathogens were detected and differentiated by using the Luminex xMAP technology, a bead array based method. The assay system was validated using constructed target sequences and viral stocks. With this method, a differential diagnosis can be made within 3 hours, and hundreds of samples can be studied per day.

It is a privilege to review the successfully completed clinical trial data, which has been submitted to the Chinese Food and Drug Administration for pre-marketing approval. To our knowledge, this is the only differential diagnostic assay under Chinese FDA consideration.(8) A total of 1066 cases were studied in a clinical trial conducted in Beijing, including 317 clinically diagnosed SARS patients; 16 suspected patients; 194 clinically ruled out patients, and 539 unaffected controls. Of the 317 clinically diagnosed patients, the molecular differential diagnostic assay identified 240 as positive for SARS, and 47 as positive for other pathogens but not SARS. All 16 suspected patients were negative for SARS, but 14 of them were detected to have other infections. One of the 194 clinically ruled out patients was tested SARS positive, and 100 among this group were positive for other infections. Finally, of the 539 controls, 52 were detected with other pathogens, but no SARS cases were detected. With this clinical data, Han and his collaborators conclude that the assay is a sensitive and accurate tool for initial screening of acute respiratory infections. The results also indicate that a significant amount of clinically diagnosed SARS patients may have other infections and were misdiagnosed as SARS patients. Also, some clinically ruled out patients may in fact have SARS. These false negative and false positive diagnoses may have severe clinical consequences.

It is critical that a quick and accurate molecular differential diagnostic assay is to give an early alert to stop the spread of SARS virus is developed. With such a tool, doctors and public health authorities could quickly identify those SARS patients that need to be isolated and treated. During the same process, the assay could also identify those with other non-SARS infections and treat them accordingly. This report is of considerable significance, because a clinical trial with 317 clinically diagnosed SARS patients may be the largest recruited number of SARS patients available, referring to the data of WHO Communicable Disease Surveillance & Response (CSR). It has posted a total of 8098 cases of diagnosed SARS and 774 deaths. The case fatality ratio of

9.6% ranges from 0 to 50% in 29 affected countries/areas as reported in the Summary of probable SARS cases with onset of illness from November 1, 2002 to July 31, 2003, revised September 30, 2003.(9)

The differential test for SARS described in the chapter of "SARS Differential Diagnosis with a Bead Array Method" of this volume is developed by a group of scientists in a US based biotech-company, Genaco Biomedical Products, Inc. This group, headed by Han, has invented several proprietary technologies that, used as a ready to go "tool box" of molecular differential diagnostics, render them the capability of developing multiplexed test in record time. For example, it took them less than 30 days to develop and validate the SARS differential diagnostic panel. This test developed by Genaco has a competitive edge over a rival test pending by Roche, because of its capacity to test simultaneously up to 17 infectious pathogens. Even without SARS, this technology is beneficial for the diagnosis of influenza and other upper respiratory infections, and to test for West Nile virus in the US, and Fever Dengue in Malaysia as well.(10-12)

The Principal Investigators conducting the clinical trials and trial sites are highly reputable. Among them, Professor John S L Tam at the Prince of Wales Hospital, Chinese University Hong Kong, is highly respected among Asian virologists, and serves the Hong Kong SAR Government as a senior consultant on SARS. Tam and his colleagues were among the earliest team to investigate SARS related viruses, and developed a plasma gene test, which takes 6 hours to further differentiate and confirm the diagnosis of SARS or Flu for patients with upper respiratory infection.

Unless hospitals are provided with approved diagnostic tests, they have to rely largely on clinical impressions and x-rays, and doctors will be forced to quarantine all patients with flu-like symptoms, stressing the facilities. Moreover, it was reported that twenty percent of infections were among healthcare workers. Not until March 2003, could RT-PCR tests identify SARS patients by multiplying SARS RNA in the lab. Unlike flu virus available in large quantity, SARS virus, in very limited quantity, is hard to trace. The improvement of the RT-PCR test has become extremely difficult, and more so now unless new SARS patients present, and with no clinical setting to prove if it works. Without a newly verified RT-PCR test, there are other options. Besides the plasma gene test by Tam and co-workers, David Perlin and his

institute are developing a rapid SARS test with molecular beacons, DNA based hairpin-like structures with tracer molecules on one end. Singapore physicians are using a PCR test developed by German biotech company Artus and US based Abbott Laboratories. Swiss based Roche says it will market a test developed with the Genome Institute of Singapore. Most viral infections like flu can, sometimes, leave people with acquired immunity, without apparent symptoms. Unfortunately, a lately laboratory report says it is "fairly unlikely" to be similar to flu. SARS seems to be a different story. Should that be the case, the next outbreak would be as severe as the first one. (8-13)

Early Detection is a priority -the first approved RNA-PCR assay in China

Early detection is a priority in the control and management of the SARS epidemic. Since SARS broke in China, Chinese scientists in academia, research institutes and biotech companies have been engaged in the development of SARS tests, including molecular diagnostic methods and serological tests. According to a News Release by the CDC AIDS Center in China, the first RNA diagnostic test for SARS was launched in the Chinese market on Oct 14, 2003.(14) On September 30, 2003, the first molecular diagnostic kit, a SARS virus RNA-PCR fluorescence test (SARS-PFDK), was approved by the China State Food and Drug Administration (SFDA). This kit, based on the real-time fluorescence PCR detection, was developed cooperatively by the Shenzhen PG biotech-company, the National Center for STD/AIDS Prevention and Control of the Chinese Center for Disease Control, and Shenzhen Donghu hospital. In one of the clinical trials in a Hong Kong Virus Unit, of the 230 stool samples from clinically diagnosed SARS patients, 145 samples (63%) were positive, and 137 of 296 plasma samples (46.2%) were positive by SARS-PFDK. All 428 samples from non-SARS patients were negative. In other sites of clinical trials, similar results were obtained.(15)

It was reported that this test detected 46.4% (13/28) stool samples and 47.4% (28/59) plasma samples from the 0-5 day onset patients. Over 50% samples were detected positive in samples from 6-10 day onset patients. This method was observed at Shenzhen PG in excellent consistency of 97.9% (47/48) in a comparison between PG and the first approved SARS diagnostic test, German ARTUS, estimating PG = 0.93(ARTUS) + 1.79 (n = 48) (15).

New SARS outbreak linked to accidental lab contamination

On September 24, 2003, a SARS outbreak in Singapore was evidently linked to an accidental laboratory contamination according to the full report released by the Singapore Ministry of Health of an international investigation of a recent SARS case. The investigation, which followed laboratory confirmation, on September 8, of SARS in a 27-year-old researcher, concluded that the patient most likely acquired the infection in a laboratory as a result of accidental contamination. It found no evidence of further transmission and no reason to regard this single isolated case as a concern for international public health. The Singapore case marked the first case of SARS to be confirmed since the last known case in the world was detected and isolated in Taiwan, China, on June 15, 2003. In addition to positive results in laboratory tests for the SARS-CoV, and subsequently confirmed by the US Centers for Disease Control and Prevention, the Singapore patient showed clinical signs consistent with a diagnosis of SARS. The patient was conducting research on the West Nile virus in a laboratory that was also conducting research using active SARS-CoV. The investigation concluded that cross-contamination of West Nile virus samples with the SARS virus in the laboratory was the source of infection. Both viruses were detected in a research specimen.(16)

Some key issues and questions related to SARS diagnosis

How many issues and questions are still under investigation? When interpreting SARS-CoV testing results, some key issues need to be answered. The CDC Fact Sheet for Clinicians seems to provide the most valuable and thorough information. It is quoted below for reference.(17) Note: information about laboratory tests for SARS-CoV and the interpretation of results from these tests is subject to change; refer to the CDC web site.

<u>What tests for SARS CoV are being done and which results are being reported?</u> At this time, tests for SARS-CoV are still being refined, and the sensitivity and specificity are uncertain and still being evaluated. It also is not known which tests perform best at which time points after onset of a patient's illness. Several types of newly developed tests are being used to test for SARS-CoV:

1. Serum antibody tests, including both enzyme immunoassay (EIA) and indirect fluorescent-antibody (IFA) formats, have been developed. State public health laboratories are using the EIA. At this time, CDC is interpreting positive test results to indicate previous infection with SARS-CoV. However, some people do not test positive until more than 28 days after onset of illness. Therefore, a negative test result can be considered a true negative only if the specimen was collected more than 28 days after the patient's onset of illness. For patients with a negative antibody test result whose specimens were obtained 28 or fewer days after illness onset, an additional antibody test should be done on a specimen drawn more than 28 days after onset to determine if they are negative or positive for SARS-CoV. The recommended timing of the second sample may be adjusted as more information becomes available.
2. Reverse transcription-polymerase chain reaction (RT-PCR) testing is also available. This test can detect SARS-CoV RNA in clinical specimens, including serum, stool, and nasal secretions.
3. Viral isolation for SARS-CoV also has been done. In these studies, clinical specimens from SARS patients are co-cultured with well-characterized cell lines, and then laboratorians look for evidence of SARS-CoV replication in these cultured cells.

The amount and type of specimens and the test type limit the number of tests that can be done. If there is sufficient specimen, both antibody testing and the RT-PCR are done. Viral isolation is the most difficult and time-consuming test and cannot be done on all patients.

What does it mean if the test results are positive for human metapneumovirus? CDC has tested some specimens from SARS patients for a variety of viruses, including human metapneumovirus. Human metapneumovirus is a recently recognized virus that belongs to the paramyxovirus family of viruses, which cause a broad range of respiratory and childhood illnesses, including mumps, measles, and croup. Human metapneumovirus is genetically related to respiratory syncytial virus, a common cause of lower respiratory tract infection in children. Several laboratories have reported positive test results for human metapneumovirus in some patients with SARS. There is not enough information to determine what role, if any, human metapneumovirus might have in some cases of SARS.

Should a patient with SARS who has negative SARS-CoV test results continue with the isolation precautions recommended by CDC and other public health authorities? As noted above, the interpretation of negative SARS-CoV test results can vary depending on which test was performed and when the testing was done. CDC advises that isolation precautions for SARS patients should be continued even if laboratory test results for SARS-CoV are negative. This recommendation is subject to change. Evaluating physicians may wish to consult their local public health authorities for advice on interpretation of SARS-CoV test results. Physicians can also check the CDC Web site for the most recent information on the interpretation of SARS-CoV laboratory results. All SARS patients should limit interactions outside the home and should not go to work, school, out-of-home childcare, or other public areas until 10 days after resolution of fever and respiratory symptoms. During this time, the infection control precautions for SARS patients should be followed.

Has the new information about SARS-CoV changed the recommendations for medical treatment for patients with SARS? The discovery that SARS-CoV is the cause of SARS has not changed treatment recommendations (see CDC's SARS website for treatment information). The new coronavirus is being tested against various antiviral drugs to see if an effective treatment can be found.

What other investigations related to SARS are planned? The state health department or CDC may contact some SARS patients regardless of whether the SARS-CoV test result was positive or negative. These patients might be asked to participate in investigations that are trying to find out more about the new coronavirus and SARS and how they are related to each other. If a patient agrees to take part in those investigations, his or her permission would be requested to collect more specimens for testing. Participation is voluntary.(17)

CDC and others have developed new tests for detecting SARS-CoV. Using these tests, CDC has identified evidence of SARS-CoV infection in several U.S. residents. Scientists from CDC and other institutions have published reports in peer-reviewed journals describing the isolation and characterization of SARS-CoV and its association with SARS. Although these reports provide strong evidence that this new coronavirus is the etiologic agent