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MINI-SYMPOSYUM: SEVERE ACUTE RESPIRATORY SYNDROME

SARS: future research and vaccine

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Summary Severe acute respiratory syndrome (SARS) is a new infectious disease of the 21st century that has pandemic potential. A novel coronavirus (CoV) was identified as its aetiological agent and its genome was sequenced within months of the World Health Organisation issuing a global threat on SARS. The high morbidity and mortality of this potentially pandemic infection demands a rapid research response to develop effective antiviral treatment and vaccine. This will depend on understanding the pathogenesis and immune response to SARS CoV. Further understanding of the ecology of SARS CoV in human and animals will help prevent future cross species transmission. Likewise for the super-spreading events, clarification of the underlying reasons will be important to prevent a large scale outbreak of SARS. Lastly it is of utmost importance that international research collaboration should be strengthened to deal with SARS and any other emerging infectious disease that can seriously threaten our future.

INTRODUCTION

Severe acute respiratory syndrome (SARS), a newly emerged infectious disease of humans in the 21st century, appeared in Guangdong Province in Southern China in November 2002 and spread to 26 countries on five continents along international air travel routes, causing large scale outbreaks in Hong Kong, Singapore and Toronto in early 2003.[1] The World Health Organisation (WHO) issued a global alert on SARS on 12 March 2003. With support of the WHO, authorities in affected regions implemented epidemiologic surveillance and adherence to infection-control procedures, including patient isolation and quarantine for contacts, which helped to contain the SARS outbreak by mid-July 2003. However, there were a total of 8098 SARS cases and 774 associated deaths.

The aetiologic agent of SARS was identified as a coronavirus (CoV)[2–5] and the genome sequence established it as a novel member of the family[6,7]. This novel CoV has satisfied Koch's postulates for causation by its consistent isolation from SARS patients, viral isolation, reproduction of disease in non-human primates after inoculation and the presence of specific antibody response against the virus in both patients and experimentally infected primates[8]. All of these remarkable findings were accomplished within a few months of the issue of the WHO global alert on SARS, testifying to the rapid response of the collaboration of the international research effort to deal with such emerging pandemics. The origin of SARS remains uncertain despite closely related CoVs that were recovered from civet cats and other animals in Guangdong Province, suggesting the SARS-CoV could have originated from such animals and implicating SARS as a zoonotic disease.[9] Other members of the CoV family can cause fatal diseases in poultry and laboratory rodents. The two previously known human CoVs cause only mild upper respiratory infections.[10]

Despite the 2002/2003 SARS epidemic being eventually controlled by case isolation, there is still neither an effective treatment for SARS nor an efficacious vaccine to prevent infection.[1] The high morbidity and mortality of SARS, as well as the potential of reemergence, make it paramount to focus on future research to develop effective means to treat and prevent the disease should it reappear. Indeed, sporadic reemergence of cases have been reported in Guangdong Province as well as from research laboratories

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in Singapore, Taiwan and Beijing since the conclusion of the 2003 SARS epidemic.

DEVELOPING A RESEARCH RESPONSE

On 30 May 2003, the National Institute of Allergy and Infectious Diseases (NIAID) convened a colloquium entitled 'SARS: Developing a Research Response' on the National Institute of Health campus, with over 500 participants that included physicians, scientists and policymakers from the United States, China, Canada, Europe and elsewhere, to coordinate a robust research response to the SARS threat in each of the following five areas: (1) Clinical research; (2) Epidemiology; (3) Diagnostics; (4) Therapeutics; and (5) Vaccines.[11] One year has gone by since this colloquium, with intense research activities conducted in many laboratories worldwide addressing those issues raised. Despite over 1900 published papers in PubMed on various aspects of SARS, many questions are still awaiting answers. This review will attempt to highlight some of the important issues that have only been partially addressed, pointing to areas of clinical interest rather than covering all aspects comprehensively.

CLINICAL RESEARCH AND THERAPEUTICS

Careful description of the clinical manifestations of SARS, correlating with virologic and immunologic parameters, has suggested that there is an initial viral replicative phase of about 10 days, possibly followed by an immunopathological phase.[12] It has been hypothesised that the immunopathological response is triggered by the viral antigens, hence the most strategic treatment is to stop the viral replication at the initial phase of the disease so that the peak viral load and the subsequent damage is minimised.[13] Therefore, an effective antiviral treatment has a window of opportunity of several days after disease onset to modify the peak viral load, thereby, in theory, decreasing the morbidity and mortality. At present, the treatment is largely empirical and therefore controversial, ranging from supportive therapy without intervention to a combination of antivirals and steroids. A recent report on an open trial of a combination of a protease inhibitor and a nucleoside analogue against a historical control suggests a favourable clinical response using lopinavir/ritonavir and ribavirin.[13] However, controversy remains as no controlled studies have been undertaken, and there is a need to set up international clinical trial networks to develop and perform clinical protocols should SARS reappear.

The future research priorities in SARS therapeutics should focus on antiviral drug screening as no clinically proven candidates exist. A high-throughput screening assay has to be developed and applied to the existing drug candidate libraries.[11] Some in vitro activity against SARS CoV was observed with certain preparations of interferon-α as well as with glycyrrhizin.[14,15] In vivo activity has also been demonstrated with interferon-α in a non-human primate model but there have been no studies in humans.[16]

Molecular studies of SARS CoV and other coronaviruses suggest several potential molecular targets for antiviral drugs. These include viral binding, fusion and other activities mediated by the glycoprotein spike on the CoV surface, as well as the RNA-dependent RNA polymerase and the cysteine protease.[11]

The identification of angiotensin-converting enzyme 2 (ACE2) as a functional receptor for the SARS CoV has opened up possibilities of treatment strategies such as interference of binding or fusion of SARS CoV with target cells.[17,18] The SARS CoV has surface spike (S) proteins which contain the S1 and S2 domains; the ACE2, which is a metallopeptidase, binds the S1 domain of the SARS CoV S protein efficiently and anti-ACE2 has been shown to block SARS CoV replication in vitro using Vero E6 cells as an experimental model.[17] A number of antibodies, peptides and small compounds can bind to ACE2 and it is possible that some of these can be useful in the treatment of SARS,[19] either by blocking the S-protein-binding site or by inducing a conformation in ACE2 that is not favourable to binding or fusion. A soluble form of the ACE2 may slow viral replication in an infected individual.[17] Recently, a human monoclonal antibody 80R, against S1 domain of the SARS CoV, has been developed that can potently neutralise SARS CoV infection and efficiently inhibit syncytia formation through blocking of receptor binding.[20] This monoclonal antibody can be used as an immediate treatment strategy for emergency prophylaxis and treatment of SARS, while the more time-consuming development of vaccines and new drugs is underway.

DIAGNOSTICS

During the winter and spring months, the diagnostic challenges of differentiating SARS from other respiratory infections can be great, making a rapid, simple and accurate diagnostic assay for SARS CoV an imperative public health tool to control any future SARS outbreak as well as the initiation of treatment protocol. The first generation PCR assays for SARS CoV were far from satisfactory but real-time PCR, coupled with an improved RNA extraction method, has allowed detection of viral RNA in nasopharyngeal aspirates with 80% specificity.[21] Knowing where in the body the virus can be found at different stages of the disease might lead to new diagnostic strategies.[22] For example, the report that SARS CoV can replicate in peripheral blood cells in SARS patients shortly after onset indicates that blood could be used as an appropriate clinical specimen for diagnosis.[23,24] Nevertheless, availability of a highly sensitive and specific direct detection method of SARS CoV in readily obtained clinical specimens such as

nasopharyngeal secretions will be most ideal, obviating the need of the laborious steps of RNA extraction.

Serologic assays are not useful for early diagnosis as IgG antibodies do not appear for 7–10 days after onset of symptoms. It has been stated that IgM antibodies typically appear earlier, but detection of IgM antibodies does not appear to permit earlier diagnosis.[1,11] Since a few SARS patients have had late seroconversion, it is best to test the convalescent serum collected at least 21 days and preferably 28 days after onset of symptoms, to rule out SARS.[1] At present, the most widely used methods for detection of antibodies against SARS CoV are indirect immunofluorescence assay and ELISA with cell-culture extract, which are difficult to standardise.[25] Therefore, recombinant-antigen-based ELISA assays are being developed using highly immunogenic nucleocapsid protein of SARS CoV, which can be used for a large scale epidemiological study of seroprevalence.[25]

IMMUNE RESPONSES AND VACCINES

The characterisation of immune responses to the SARS CoV, including the impact of SARS on immune function and any immunopathological responses the virus may trigger, is crucial in helping the development of new therapeutic strategies and vaccines.[11] There is a rapid and generalised lymphopaenia in patients with SARS during the acute phase of infection, which is in distinct contrast to the proliferative response seen in HIV-, CMV- or EBV-infected patients.[26] In patients who recovered from SARS, an equally rapid and dramatic restoration of T cell, B cell and NK cell counts was seen in peripheral blood. The mechanism through which the SARS CoV precipitates such lymphopaenia so rapidly is unclear but could be related to immunologic trigger of apoptosis of uninfected lymphocytes. Elucidation of the underlying mechanism may help design treatment strategies.

The initial increase in viral load in the first 10 days of disease[12] also suggests that the role of innate immunity as a first-line defence is important and may influence the subsequent disease progression. It is important to identify the host innate immune response genetics that are predictors of disease susceptibility and progression. This would help to improve prognostic capabilities and allow identification of patients likely to benefit from aggressive interventions. Such information is also of value in the development of entry criteria for interventional studies.[11]

Since immune response might play a role in SARS pathogenesis, one must be cautious of the possibility of enhancement of the disease in immunised subjects.[27] This has indeed occurred for experimental vaccine directed against feline infectious peritonitis virus, which is also a coronavirus. Therefore caution has been urged on developing SARS vaccines.[27]

However, given the urgent need for a safe and effective SARS vaccine, multiple strategies for vaccine development have been pursued simultaneously. At least 10 candidate SARS vaccines are at different phases of development.[27–31] These include the development of live-attenuated and inactivated virus vaccine, in addition to other strategies that elicit strong T cell responses, such as DNA-based vaccine and engineered adenovirus vectors, as well as those that elicit production of neutralising antibodies.[11] Subunit vaccines based on S protein fragments and peptides are also being developed. SARS CoV S protein, expressed by attenuated vaccinia virus, has been shown to immunise mice protectively.[30] Similarly, a DNA vaccine encoding the SARS CoV S protein can induce T cell and neutralising antibody responses, as well as protective immunity, in a mouse model.[31] Viral replication can be reduced by more than six orders of magnitude in the lungs of mice vaccinated with these S plasmid DNA expression vectors and protection is mediated by a humoral but not a T-cell-dependent immune mechanism.[31]

All the experimental testing of candidate SARS vaccines would require an animal model that reproduces SARS symptoms and pathology as in humans. However, no animal model described to date can reliably mimic the respiratory symptoms seen in humans with SARS.[27] Even the macaques model, used initially to fulfil the last of Koch's postulates in confirming SARS CoV as the etiologic agent,[8] does not always reproduce the SARS-like symptoms when infected with SARS CoV.[27] This could be due to macaques not being inbred like mice. Finding a consistent SARS animal model which can be used for testing potential drugs and vaccines is certainly a top research priority. Monkey, mouse and ferret have all been used as animal models for answering different research questions.

ECOLOGY AND EPIDEMIOLOGY

The origin of SARS CoV and its ecological relationship with other animal CoV will certainly be an important topic of research, as the animal reservoir of such CoV will be the constant potential source of another SARS outbreak. Understanding of the ecology will help to implement measures to minimise transmission across species taking place again. Nevertheless, the recent sporadic reemergence of SARS was due to lapses of security in research laboratories in handling SARS CoV, testifying to the urgent need to maintain and monitor laboratory safety in research institutes that deal with such pathogens.

Another mystery in the 2003 SARS epidemiology is the so-called super-spreading events, such as the community outbreak in the Amoy Garden housing complex of Hong Kong, which affected more than 300 residents of this private housing estate.[32] Tentative evidence of airborne transmission of the SARS CoV has been suggested by an epidemiologic analysis of this outbreak, coupled with airflow simulations and experimental studies.[32] Better understanding of these super-spreading events will be crucial in preventing similar events from happening again.

THE FUTURE

Sporadic cases of SARS may continue to appear but if vigorous public health measures can be maintained another major outbreak is unlikely, making it impossible to perform controlled clinical studies of either candidate antivirals or vaccines for SARS CoV.[27] Nevertheless, the global community needs to be prepared for such emerging and reemerging infectious diseases if the human race is to have a future.

RESEARCH DIRECTIONS

- Understand the pathogenesis and immune responses to SARS CoV.
- Develop effective antiviral therapeutics and efficacious vaccines.
- Develop rapid and accurate diagnostic tests for early diagnosis of SARS CoV infection.
- Clarify the ecology of human and animal SARS CoV to prevent cross species transmission.
- Understand the reasons for super-spreading events.

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FURTHER READING

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