Severe Acute Respiratory Syndrome – a New Coronavirus from the Chinese Dragon's Lair*

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Abstract

The recent identification of a novel clinical entity, the severe acute respiratory syndrome (SARS), the rapid subsequent spread and case fatality rates of 14–15% have prompted a massive international collaborative investigation facilitated by a network of laboratories established by the World Health Organization (WHO). As SARS has the potential of becoming the first pandemic of the new millennium, a global warning by the WHO was issued on 12 March 2003. The disease, which is believed to have its origin in the Chinese Guangdong province, spread from Hong Kong via international airports to its current worldwide distribution. The concerted efforts of a globally united scientific community have led to the independent isolation and identification of a novel coronavirus from SARS patients by several groups. The extraordinarily rapid isolation of a causative agent of this newly emerged infectious disease constitutes an unprecedented scientific achievement. The main scope of the article is to provide the clinician with an overview of the natural history, epidemiology and clinical characteristics of SARS. On the basis of the recently published viral genome and structural features common to the members of the coronavirus family, a model for host cell–virus interaction and possible targets for antiviral drugs are presented. The epidemiological consequences of introducing a novel pathogen in a previously unexposed population and the origin and evolution of a new and more pathogenic strain of coronavirus are discussed.

Introduction

In the fall of 2002, the first reports of 305 cases of a highly contagious and severe atypical pneumonia, the severe acute respiratory syndrome (SARS, see Table 1 for case definitions), emerged from the Guangdong province of southern China. By mid-February 2003, the first cases were found in Hong Kong. On March 12, the disease spread outside China, causing the World Health Organization (WHO) to issue a global alert. Subsequent spread of the virus by international air travel has led to disease outbreaks world wide. Daily updates and case counts are available on the Internet at the WHO website (http://www.who.int).

Distribution and spread

At present, SARS has been found in 32 countries (see Fig. 1) with a total number of probable cases amounting to 8295. The climbing death toll is currently 750 (as of May 29). Many countries appear to have succeeded in containing the disease and preventing further spread. Vietnam was the first country to report that no new cases had been found for 20 days (three times the mean incubation period [1]), thus removing Vietnam from the list of countries with local transmission of the disease. In China, however, the situation remains disturbingly out of control. WHO epidemiologists have recently gained access to military compounds housing SARS patients, and in collaboration with the Chinese health authorities they are trying to establish procedures for assessment and containment of the disease. In accordance, the latest reports from Beijing and the Guangdong province show that the number of new cases may have begun to decline. However, WHO officials have cautioned against any clear conclusion that the SARS outbreak in China has peaked, as many factors that influence the outbreak's future evolution remain unknown. For example, half of all new probable cases in Beijing do not have any recorded previous contact with a SARS patient [2]. A study by Donnelly *et al.* [1], including 1425 cases

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Table 1 Severe acute respiratory syndrome (SARS) case definitions (World Health Organization criteria)

Suspect case

1. A person presenting after 1 November 2002 with history of:
- high fever (>38 °C)

AND
- cough or breathing difficulty

AND one or more of the following exposures during the 10 days prior to the onset of symptoms:
- close contact with a person who is a suspect or probable case of SARS
- history of travel to an affected area
- residing in an affected area

2. A person with an unexplained acute respiratory illness resulting in death after 1 November 2002, but on whom no autopsy has been performed

AND one or more of the following exposures during to 10 days prior to onset of symptoms:
- close contact with a person who is a suspect or probable case of SARS
- history of travel to an affected area
- residing in an affected area

Probable case

A suspect case with
- radiographic evidence of infiltrates consistent with pneumonia or respiratory distress syndrome (RDS) on chest X-ray (CXR)

OR
- autopsy findings consistent with the pathology of RDS without an identifiable cause

Exclusion criteria

A case should be excluded if an alternative diagnosis can fully explain their illness

from Hong Kong, has investigated the key epidemiological determinants for the spread of the SARS virus: infection to onset, onset to admission, admission to death, and admission to discharge. The mean incubation period was estimated to be 6.4 days (range 2–16 days), and the mean time from symptom onset to hospital admission was 3–5 days.

The first cases of SARS in Scandinavia have recently been described in Sweden and Finland, and in view of the increasing international travel rates imported cases will undoubtedly occur in the future. In accordance with this, the infectious disease departments at Copenhagen University Hospitals have adopted strict isolation procedures, and an

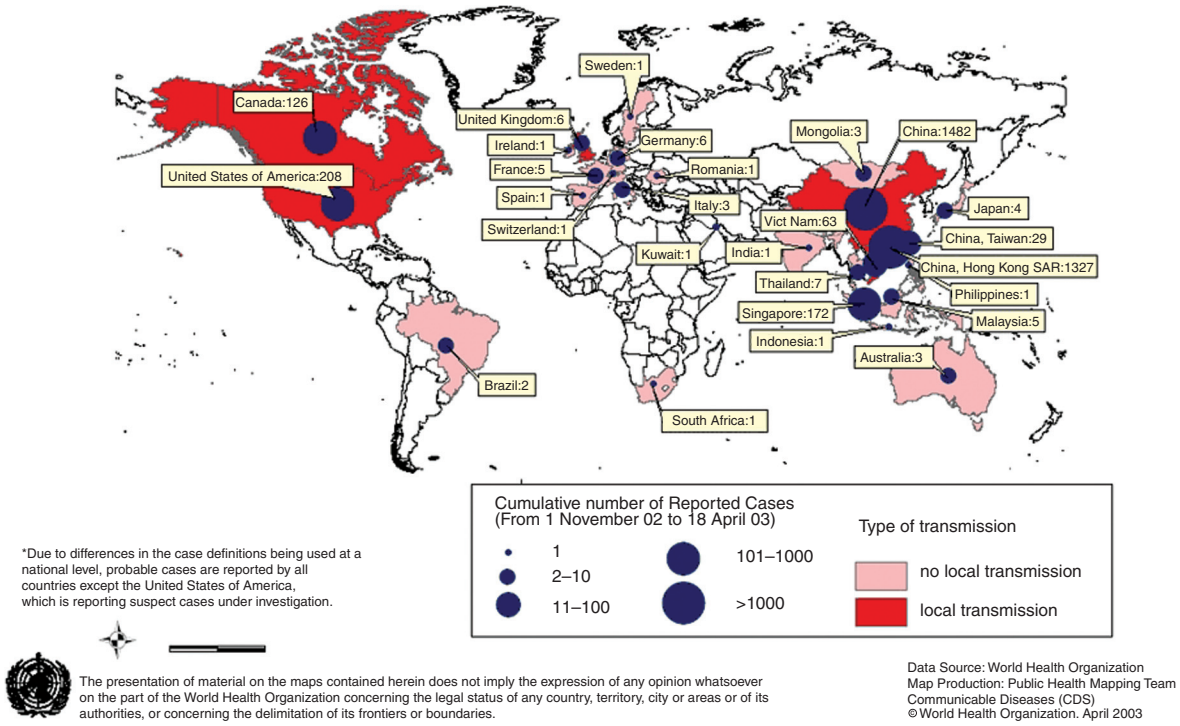

Figure 1 Severe acute respiratory syndrome (SARS): cumulative number of reported probable cases. This figure has been reproduced from the World Health Organization (WHO) website (http://www.who.int/csr/sars/map2003).

emergency response plan to contain a potential outbreak has been devised and implemented. No secondary cases of SARS have been reported in Scandinavia.

Clinical features

As the number of patients with SARS is accumulating, the clinical features of the disease are becoming more and more evident (summarized in Table 2) [1, 3–5]. Peiris *et al.* [5] found that fever and pneumonia initially responded to treatment with ribavirin and corticosteroids, but after a week (7–9 days) patients subsequently developed relapse of fever (85.3%), radiological deterioration (80%), watery diarrhoea (73.3%) and respiratory deterioration (45.3%). Twenty per cent progressed to acute respiratory distress syndrome (ARDS) during the third week. Age and Hepatitis B status were found to be independent risk factors for progression to ARDS [1, 5]. Estimates based on a parametric γ distribution yielded case fatality rates of 13.2% and 43.3% in patients below and above 60 years of age, respectively, assuming a nonparametric method corresponding rates were 6.8% and 55% [1]. The overall case fatality rate is 14–15% [6].

Identification of the infectious agent

The concerted efforts of a globally united scientific community have led to the independent isolation and identification of a novel coronavirus from SARS patients by several groups [7–10]. The extraordinarily rapid isolation of a causative agent of this newly emerged infectious disease constitutes an unprecedented scientific achievement.

The nature of this pathogen has been elucidated using a wide array of techniques such as: virus isolation and characterization by electron microscopy, reverse transcriptase-polymerase chain reaction and serological tests [7, 8]. Clinical specimens from patients with SARS have been searched for unknown viruses with the use of cell cultures

Table 2 Clinical features of severe acute respiratory syndrome (SARS)

Incubation period
The mean incubation period is 6.4 days but varies from 2 to 16 days.

Duration
Mean viraemia levels peak on day 10 and drops to admission levels on day 15 and mean length of hospital stay is 22 days.

Symptoms
The most common symptoms can be summarized as follows:
- ~100% had fever (temperature >38 °C for over 24 h)
- ~70% had chills and/or rigor
- ~60% had myalgia
- ~50% had cough and/or headache
- ~40% had dizziness

Less common symptoms (<30%) include sputum production, sore throat, coryza, nausea/vomiting and diarrhoea

Clinical signs
- ~90% had crackles on auscultation
- ~70% had dullness on percussion

Paraclinical findings
- ~70% had lymphopenia and/or increased lactate dehydrogenase levels
- ~45% had thrombocytopenia, prolonged activated partial thromboplastin time (APTT) and/or elevated D-dimer levels

Less common findings (<35%) include increased levels of alanine aminotransferase (ALAT) and creatine kinase as well as hypokalaemia and hyponatraemia

Diagnostic imaging
Serial chest X-rays may show progressive lung infiltrates
Pulmonary CT-scans may show ground-glass appearance, diffuse or patchy consolidation but no interstitial pattern

Diagnosis
World Health Organization criteria for suspected case and probable case respectively. Confirmation should be sought by polymerase chain reaction amplification of the SARS-related coronavirus in relevant specimens

Severity, clinical course and prognosis
- ~15% dies
- ~20% develop ARDS

Increasing age is the most important risk factor for the development of severe disease as well as for high mortality rates

Treatment
Experimental treatments include corticosteroids and ribavirin

Prophylaxis
Prophylactic measures include the use of mask with virus filter, gown, gloves and eye protection. Isolation procedures in low pressured rooms with double doors

and molecular techniques. The identified coronavirus was isolated in cell culture, and a sequence of 300 nucleotides in length was subsequently obtained by a polymerase chain reaction (PCR) random amplification procedure. On the basis of the obtained sequence, conventional and real-time PCR assays for specific and sensitive detection of the novel virus have been established. Subsequent detection of the virus in a broad range of clinical specimens from patients fulfilling the clinical case definition, but not in controls, has been used to validate the detection assays. Virus from sputum samples has been able to infect and exhibit cytopathic effect *in vero* E6 cells [7, 8]. In addition, the presence of virus has been demonstrated in a suspension of kidney tissue obtained by autopsy, and serologic evidence of virus antibodies has been demonstrated in serum and faeces obtained during the convalescent phase. The above findings and the recent demonstration that SARS virus fulfils Koch's postulates [11] lead to the conclusion that SARS is caused by a novel 29 kb single-stranded RNA virus, either alone or as a coinfection with another infectious agent.

Origin and modes of transmission

SARS virus is thought to have evolved through zoonosis as a consequence of Chinese agricultural tradition, where many types of domestic and wild animals are kept closely together allowing viral propagation outside the natural host. At this point, preliminary studies have implicated a number of wild animals as the possible natural reservoir. A joint study by research teams in Hong Kong and Shenzhen, China has reported the presence of several coronaviruses closely related genetically to the SARS virus in two of the animal species tested (masked palm civet and racoon-dog). The study also found that one additional species (Chinese ferret badger) elicited antibodies against the SARS coronavirus. These and other wild animals are traditionally considered delicacies and are sold for human consumption in markets throughout southern China [12].

The primary mode of transmission of SARS is through droplets and indirect or direct contact. At present, there is no conclusive evidence suggesting airborne transmission. Virus particles have been shown to be fairly stable, remain infectious for up to 48 h after drying on plastic surfaces and possibly up to 4 days in faeces from patients with diarrhoea [13]. These findings may partially account for some of the observed clusters of outbreaks, as 66% of the Amoy gardens patients presented with diarrhoea compared with only 2–7% in other local outbreaks [13]. Case clusters seem to have played a key role in the early stages of the epidemic [1], and the possible existence of so-called *super-spreaders* (individuals causing an unusual large amount of secondary cases) has been the subject of some speculation. These would explain some of the apparent disproportionate features observed when tracking disease spread; however, little evidence has been produced in support of this hypothesis.

Patterns of disease progression

Virus has been detected in high concentrations in sputum (100 million molecules per millilitre), and in lower concentrations in blood and faeces during both acute and convalescent phases. Viral load peaks on day 10 and decreases to admission level on day 15 [5]. The clinical characteristics of the disease likewise suggest a predilection for the respiratory epithelium of upper and lower airways (i.e. fever, dry cough, dyspnoea and hypoxia). Paraclinical findings include air space shadowing predominantly in lower lung zones on chest X-rays and CT scans, consistently excluding interstitial patterns. The characteristic finding on CT was bilateral peripheral air-space ground-glass consolidation mimicking bronchiolitis obliterans with organizing pneumonia [3, 8, 14, 15]. In close to half of the cases, the resolvement of pulmonary infiltrates is associated with the appearance of new radiological lesions at other sites. Histological examination of lung tissue from fatal cases show diffuse alveolar damage similar to that observed in early ARDS [3, 16]. Pathological changes include epithelial cell proliferation, the presence of giant cell infiltrates and an increase in pulmonary macrophages. Haemophagocytosis suggestive of cytokine dysregulation has been demonstrated in a subset of patients [16, 17].

The temporal patterns of peak viraemia, radiologic deterioration and pathological changes indicate that immunologically mediated tissue damage may account for the clinical severity of the disease.

The Coronaviridae

The Coronaviridae consists of two genera: coronavirus (serogroups I, II and III) and torovirus, both containing human pathogens. In humans, the corona virus is a frequent cause of the common cold presenting as coryza, cough and general malaise. Numerous biologically distinct avian and mammalian coronaviruses have been isolated. Different strains have the ability to genetically recombine [18]. Phylogenic analysis of the SARS genome and of representatives of the three known groups of coronaviruses revealed that the proteins encoded by the SARS virus do not cluster within any of the three serogroups [7, 8, 19, 20]. SARS virus is most closely related to the group II family of coronaviruses with whom it shares 50–60% nucleotide sequence homology. It has been suggested that SARS virus can be ascribed as the first representative of a possible fourth serogroup of coronavirus [19]. Coronaviruses are large RNA viruses characterized by a distinctive morphology (Fig. 2A), replication strategy, genomic organization (Fig. 2B), and sequence homology (Fig. 2C). Nearly all known coronaviruses are host specific, although a few strains are capable of crossing the species barrier (e.g. the bovine coronavirus which can infect several species both *in vitro* and *in vivo*) [18]. SARS virus can enter and replicate in both African Green Monkey kidney (vero E6) cells

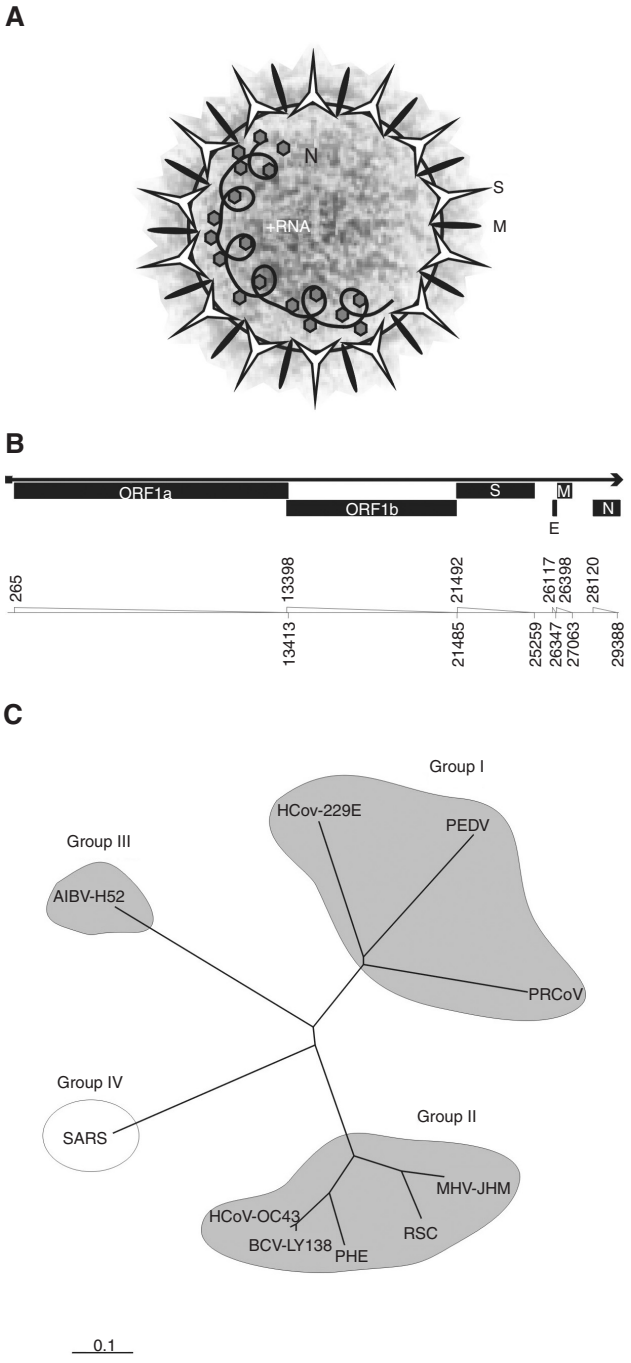

Figure 2 (A) Model of SARS structure. SARS virions are round, moderately pleomorphic enveloped particles, measuring 100–150 nm in diameter and covered with a distinctive fringe of widely spaced, club-shaped surface structures about 20 nm in length composed of the spike glycoprotein (S). The membrane glycoprotein (M) interacts with the nucleocapsid. The viral nucleocapsid consist of the 29.7 kb plus-stranded genomic RNA and the capsid phosphoprotein (N). (B) Genomic organization of SARS. Overall organization of the 29.7 kb genomic RNA. Predicted open reading frames (ORF) 1a and 1b encode the RNA polymerase and nonstructural proteins, followed by S, E, M and N. Numbers above line indicate the start position of the ORF, and number beneath the line indicate the stop position of the ORF. The numbering is based on the genome of SARS virus deposited in GenBank, accession number NC_004718. (C) Phylogenic dendrogram of coronavirus spike glycoproteins. The length of each branch depicts relative sequence homology between the proteins. Shaded areas illustrate receptor clusters. Abbreviations are severe acute respiratory syndrome virus (SARS), human coronavirus strain 229E (HCoV-229E), porcine epidemic diarrhoea virus (PEDV), porcine respiratory coronavirus (PRCoV), avian infectious bronchitis virus strain H52 (AIBV-H52), mouse hepatitis virus strain JHM (MHV-JHM), rat sialodacryoadenitis coronavirus (RSC), porcine haemagglutinating encephalomyelitis virus (PHE), bovine coronavirus strain LY138 (BCV-LY138) and human coronavirus strain OC43 (HCoV-OC43). The phylogenetic tree is based on a CLUSTAL X 1.81 alignments of the full amino acid sequence of all included spike proteins and the graphic presentation were generated using TREEVIEW.

[7, 8, 19] and in fetal rhesus kidney cells (FRhK-4) [21]. This observation suggests that SARS could be unusually indiscriminate regarding target cells and organisms.

Structure and morphology

Coronaviruses are approximately 100–150 nm in diameter and consists of a host cell-derived envelope with 20 nm complex surface projections surrounding the periphery and a helical nucleocapsid containing the viral RNA sense-stranded genome (Fig. 2A). The prefix corona is derived from the 'crown-like' appearance of the envelope proteins seen in the electron microscope. Coronaviruses have the largest genomes of all known RNA viruses (27–32 kb). The genome encodes an RNA-dependent RNA polymerase and four structural proteins, which are shared by all coronaviruses. The structural proteins include three surface glycoproteins – the spike (S), envelope (E) and membrane proteins (M) – as well as the capsid phosphoprotein (N), which encapsulates the RNA sense strand (Fig. 2A). The

genetic order of the polymerase and the four structural proteins is pol-S-E-M-N [18, 20] (Fig. 2B). The surface protein haemagglutinin esterase is only found in certain strains in serogroup II and is not present in SARS virus [7, 8, 19, 20]. The genome also encodes a number of non-structural proteins that show great variation between different strains of coronaviruses. Their function is unknown and they are not essential for viral replication. The SARS virus genome likewise encodes seven potential nonstructural proteins that do not show significant sequence similarity with any known proteins in preliminary studies [20].

Viral entry and replication of coronavirus

The life cycle of coronavirus is well established [18] and may serve as a model for SARS. The first step in the viral life cycle of coronavirus consists of the binding of virions to the plasma membrane of susceptible cells through interaction between the viral S protein and specific host cell receptors (e.g. the human amino peptidase receptor) initiating binding, fusion, penetration and uncoating. As for all plus-stranded RNA viruses, the first synthesis following entry is the translation of the viral genomic RNA to yield an RNA-dependent RNA polymerase. The plus-stranded genomic RNA is then transcribed into minus-strand RNA, which is used as template for the synthesis of plus-stranded viral mRNAs and of genomic RNA. The first 60% of the length of the genome from the 5′ end (approximately 20 kb) consists of two overlapping open reading frames ORF1a and ORF1b, which encode the viral RNA-dependent RNA polymerase and other non-structural proteins. These include a papain-like protease, a 3C-like protease and a helicase. A ribosomal frameshift mechanism initially translates ORF1a and ORFb into a large polyprotein, which is subsequently processed into multiple proteins by protease cleavage. N is translated on free polysomes, whereas both M and S are inserted into the rough endoplasmic reticulum (RER) and transported to a Golgi-associated complex [18]. Also, a fraction of S is targeted to the plasma membrane, where it plays a role in cell–cell fusion. In brief, the formation of virions includes three steps: (1) binding of nucleocapsid to intracellular membranes that contain the M protein, (2) spike formation by the incorporation of S protein from a Golgi-associated compartment at the time of budding and (3) release of virions by the budding of large intracellular vesicles [18].

Targets for intervention

As SARS has molecular mechanisms that are different from human, these are obvious targets for antiviral intervention. Increased knowledge of key events in the replicative cycle of SARS virus may thus facilitate the development of new antiviral drugs. The SARS virus entry receptor has not yet been found, but some coronaviruses, including the serogroup I coronavirus 229E, enter target cells by the binding of the S protein to human aminopeptidase N (CD13) [22]. *In vitro*, binding can be inhibited using antibodies against CD13, by soluble CD13 receptor and specific inhibitors of CD13 [23]. Analogous to the use of reverse transcriptase inhibitors in HIV infection, the RNA-dependent RNA polymerase is an obvious target of intervention as it is not naturally present in human cells. Ribavirin, an RNA-nucleoside analogue that is effective in some cases of hepatitis C infection, has been tried in SARS-infected individuals [7, 8]. However, no effect on the clinical outcome of SARS has been shown. The papain-like protease, 3C-like protease and helicase found in coronaviruses also offer possible targets for the introduction of SARS-specific protease inhibitors. Based on the analysis of the crystalline structure of the coronavirus (strain 229E) 3c-like protease, Anand *et al.* [24] have proposed that the available rhinovirus $3C^{pro}$ inhibitors may be modified to make them useful for SARS therapy.

There has been no attempt to develop a vaccine against coronaviruses in humans. In contrast, commercially available vaccines based on inactivated or attenuated coronaviruses can induce neutralizing antibodies in the peripheral blood of immunized animals, but they only provide limited protection for the disease [25]. Serum antibodies against S, M and N proteins are produced in adult volunteers in response to inoculation and infection with human coronaviruses [26]. Infection of adult volunteers with 229E-like coronavirus has been shown to confer immunity on rechallenge with the same strain 1 year later. However, infection with other 229E-like coronavirus strains produced infection and illness [27]. It should be noted that all volunteers had antibodies against coronaviruses prior to infection with the 229E-like strain, reflecting that coronavirus infection is common in humans [28]. As SARS has not previously infected humans, the lack of acquired immunity may increase the virulence and spread of the virus. Serum from SARS-infected survivors who cleared the infection has neutralizing antibodies against the virus [5], indicating that a potential vaccine may be efficient. It is unclear whether an effective vaccine against SARS can be made using classical vaccine methods (recombinant proteins or inactivated or attenuated viruses) and whether cell-mediated response is necessary for immunity. In all cases, the search for a vaccine is most warranted.

SARS is not the only coronavirus to cause pulmonary disease in humans. Previous studies have reported severe lower respiratory disease among frail elderly people [29], pneumonia among all age groups [30, 31] and acute lower respiratory tract disease in infants [32] to be caused by coronavirus. In none of these studies has coronavirus been associated with significant mortality. Apparently, SARS does not cause as severe disease in children as in adults [1, 33]. Furthermore, the temporal pattern of clinical and

radiological progression, the time of peak viraemia and pathological findings [17] suggest that the observed deterioration during the second week may be because of immunologically mediated tissue damage rather than uncontrolled viral replication. These findings have led to the inclusion of corticosteroids in experimental protocols, as these have proved to be beneficial in certain cases of ARDS [34, 35]. It could be speculated whether the damage inflicted by SARS could be caused by an adverse human immune response, thereby making infection at a young age an advantage. A distinguishing feature of SARS virus may be the ability to elicit an adverse immune reaction – a theory, which should spur efforts to further characterize the known immunogenic epitopes like the S protein and intensify the search for possible new antigens encoded by the SARS viral genome.

Perspectives

At present, it is impossible to forecast whether the continuing efforts to contain and eradicate SARS will succeed. However, the swift reaction from a globally united scientific community has secured a rapid identification and characterization of the pathogen causing SARS and has provided essential diagnostic tools at a staggering pace. The existing body of knowledge on coronaviruses presents scientists with a multitude of potential targets for medical intervention. In addition, the strategy for containment outlined by WHO has proved to be effective in industrialized as well as Third World settings. The question remains whether the disease is by now too far advanced in China to be contained. The upcoming season for dengue and influenza virus is bound to interfere with the rather vague clinical case definition and may render the present strategy for containment inadequate by exceeding the capacity of the Chinese healthcare system. Other coronaviruses are epidemic in nature [18] and if SARS is not eradicated, a similar pattern of cyclic outbreaks may be anticipated. There are numerous examples of the epidemiological consequences of introducing a novel pathogen in a previously unexposed population. If SARS becomes endemic, the development of immunity in the affected population may eventually decrease the mortality; however, there are a number of discomforting characteristics of this new pathogen. With the notable exception of acquired immune deficiency syndrome, the majority of emerging infectious diseases since the 1980s have exhibited features limiting their capacity to pose major threats to international public health (e.g. West Nile fever requires a mosquito vector, Creutzfeldt–Jakob disease depends on food as a vehicle for transmission and Ebola/Marburg viruses render their host visibly ill and too unwell to travel). However, a distinguishing feature of SARS appears to be the absence of factors limiting disease spread. The consequences of SARS gaining foothold in densely populated countries with poor infrastructure like India and in areas with immunocompromised populations as seen in sub-Saharan Africa could thus be grave.

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