SARS CORONAVIRUS: AN OVERVIEW

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Summary

In recent years, emerging viral infections have become a serious problem. Severe acute respiratory syndrome (SARS) represents the first transmissible disease of the 21st century. The dramatic chain of transmission brought to the world's attention this new respiratory disease and it clearly illustrated the potential for SARS to spread extensively from a single infected person and to rapidly disseminate globally. The identification of the aetiological agent, SARS-associated coronavirus led to a series of decisive and effective containment efforts and to a new vaccine.

Key Words: SARS, Coronavirus, vaccine

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SARS epidemiology

SARS is a febrile respiratory illness caused by a new coronavirus (SARS-Cov) (1), a RNA-genome based virus. SARS-Cov while evolves consistently and rapidly within its animal and human hosts, both the infectivity of the virus and the severity of the disease modify along with the variation/adaptation of the virus to its hosts (2). The pandemic started in the Guangdong Province of China, and since February 2003 had spread to Hong Kong and subsequently to 32 other Countries or regions worldwide, infecting over 8,000 patients and resulting in 774 deaths (3). The overall mortality during the outbreak has been estimated at 9.6%. The overriding clinical features of SARS are the rapidity with which many patients develop the symptoms of acute respiratory distress syndrome (ARDS). This complication occurs in approximately 16% of all SARS patients, and when it occurs is associated with a mortality rate of 50% (4).

The epidemiology of SARS outbreaks suggests that SARS-Cov is transmitted primarily through droplets and close contacts between individuals. Studies documenting the stability of the virus for days in the environment raise the possibility of fomite transmission, and in a few instances, transmission by small-particle aerosols can not be excluded. In particular, aerosol-generating medical procedures (e.g. endotracheal intubation, bronchoscopy) may be associated with an increased risk of transmission in health care settings (5,6). Given that profuse watery diarrhoea is seen in a significant proportion of patients and SARS-Cov can be identified in large quantities in stool, faeces remain a possible source of virus and faecal-oral or faecal-respiratory spread are the leading hypotheses for large outbreaks (7).

SARS-Cov genome contains five major open reading frames (ORFs) encoding the replicase poly-protein, the spike (S), the envelope (E), membrane (M) glycoproteins and the nucleocapsid protein (N) in the same order and of approximately the same size as those of other coronaviruses. ~~e~~ Coronaviruses, together with the *Arteriviridae* and *Roniviridae*, belong to the order *Nidovirales* (8). The arteriviruses and coronaviruses can cause enteric and respiratory tract infections in mammals and birds while the roniviruses infect fish. The severity of pathogenesis varies depending on the viral genotype. Soon after SARS-Cov was identified as the causative agent of SARS, antiviral screening programs have been initiated. These programs have identified several antiviral agents that inhibit SARS-Cov replication *in vitro*. These results led to the experimental use of protease inhibitors and interferon alpha (IFN-α) in the treatment of patients.

Aetiological Agent

SARS-Cov is an enveloped, positive-stranded RNA virus in the Coronaviridae family. Coronaviruses are associated with a variety of enteric and respiratory disease syndromes in several animal species.

Successful infection of host cells requires many steps that are common to all coronaviruses. SARS-Cov binds to host cells *via* a specific SARS receptor, angiotensin-converting enzyme 2 (ACE-2) (9,10). Following the entry, the virus uncoats, DNA is released, and transcription occurs followed by the production of viral proteins.Viruses by definition are dependent on the host cellular machinery for replication. However, as the viral lifecycle progresses inside the cell, the virus not only utilizes certain host enzymes, but it also generates proteins that are not available in the host repertoire. Many coronavirus proteins are translated from subgenomic RNAs (sgRNAs) rather than the genomic RNA (11). This causes the translation of plus-strand viral message RNA into proteins that theoretically occurs in at least two phases: first the genomic RNA serves as a template for production of non-structural proteins including RNA-dependent RNA polymerase (RDRP); then RDRP uses genomic RNA as a template for the production of sgRNAs. Structural and accessory proteins are produced from the sgRNAs in the next phase of translation. Thus, the second phase of translation cannot occur without the production of enzymes from the first phase. It is not known whether successful infection requires the presence of more than one copy of the genomic RNA. The search for the origins of SARS-Cov and its potential reservoir(s) is in progress. As many as 42% of the early SARS cases in Guandong occurred among people who were involved in animal trade or in food preparation, (7,12). Individuals involved in animal trade in Guandong were more likely to have antibodies to SARS-Cov than those who did not trade animals or general population controls. In addition, a coronavirus with 99% homology with human SARS-Cov isolates was recovered from civet cats and from a racoon dog sold alive as food in markets in Guangdong. Collectively, these observations support the hypothesis that SARS-Cov was first transmitted from wild animals used as food for humans, with subsequent person to-person transmission. Experimental studies have shown that ferrets and domestic cats are also susceptible to infection by SARS-Cov and that they can efficiently transmit the virus to previously uninfected animals that are housed with them. This finding suggests that SARS may have been transmitted periodically in the past from animals to humans and the virus may have adapted to humans (13) or that occasional events resulted in an efficient transmission that led to the outbreak in 2003.

SARS Immunity

The site of initial infection with SARS-Cov is not known as well as the pathogenesis of SARS is not completely understood. Angiotensin-converting enzyme 2 (ACE2) is efficiently expressed in lung, heart, kidney, and gastrointestinal tissue in humans. SARS-Cov RNAbis presented in the plasma and serum of more than 50% of patients during the first week of illness (14).

Pathological samplings of the lungs of SARS patients during the first 10 days of illness include pneumocyte proliferation and desquamation, hyaline membrane formation, mixed inflammatory infiltrate, and intra-alveolar oedema (7,15). Increased numbers of interstitial and alveolar macrophages, with focal haemophagocytosis in interstitial macrophages, have also been described. In cases of prolonged duration, diffuse alveolar damage, squamous metaplasia, and multinucleated giant cells, of macrophage origin, are also observed. During SARS-Cov infection, the immunity was characterized as lymphopenia, specific antibody production, cytokine profile and specific responses to individual viral proteins. IFN-γ, a Th1 cytokine which is associated with potent cell-mediated immunity and resistance to intracellular pathogens, increased dramatically during SARS-Cov infections, while IL-4, the dominant Th2 cytokine, which promotes humoral immunity that protects against extracellular microbial infections, decreased after SARS-Cov infection. This suggests that Th1 dominated-responses cleared the viral infection from the body. However, another Th2 cytokine, IL-10 was also elevated in SARS patients. Mainly, IL-10 is produced by Th2 that has a dual effect on T lymphocytes in terms of inhibiting Th1 cells to produce IL-2 and interferons as well as tumor necrosis factor (TNF). It also promotes the proliferation and cytolysis activity of CD8 and NK cells. Therefore, it is possible that elevation of IL-10 expression is associated with the susceptibility to the disease. As for IL-2 expression, Li et al. (16) and Duan et al. (17) suggested the presence of a high level of expression after SARS infection whereas others did not (10,18). Expectedly, inflammatory cytokines are also elevated dramatically. He et al. (19) showed that comparing total lymphocyte counts from SARS patients with those from control individuals a significant decrease in CD45+, CD3+, CD4+, CD8+, CD19+ and CD16/56+ counts, over each of the five weeks of the SARS illness. However, CD4+/CD8+ ratio did not change significantly over the course of the illness. The various lymphocyte populations (CD45+, CD3+, CD4+ and CD8+) were below the normal ranges in the first week of the clinical illness, reaching a peak during the second week before returning to normal levels.

Lymphopenia is a very common feature for SARS infection (10,20). According to Wong's report (21), about 98% of the patients had lymphopenia during their course of illness. Most patients had normal lymphocyte counts at the onset of disease. Progressive lymphopenia occurred in the early course of illness and reached its lowest point in the second week in most cases. While CD4 T lymphocytes counts are reduced in nearly all SARS patients, reductions in circulating levels of CD8 T lymphocytes, B lymphocytes, and natural killer cells are also a common feature (21). Thus, in SARS infection, lymphopenia reflects the severity of infection and may be a good marker of disease activity. Death from SARS usually occurs late in the course of illness (1 week after onset) and has been attributed to adult respiratory distress syndrome, multi-organ failure, thromboembolic complications, secondary infections, and septic shock. Some investigators proposed that depletion of lymphocytes was due to apoptosis (22). Another explanation for the lymphopenia is that lymphopenia occurs when the body's mechanisms for down-regulation of lymphocyte differentiation, particularly mediated by IL-10 from the cytokine cascade, occur. It may also be hastened by down-regulation following infection and activation of T lymphocytes.

Control Strategies

Influenza is transmitted through close contacts *via* large droplets, direct or indirect. Transmission by fine droplet inhalation may also occur (23). Adults infected by seasonal influenza virus are typically infectious at/or before the onset of illness.Children and immune-compromized individuals can shed the virus for longer periods of time than non immune-compromized adults. Contagiousness of a virus varies inversely with the level of immunity in the population (24). Thus, the containment or attenuation of pandemic influenza before the availability of an effective vaccination depends on early detection and before large numbers of individuals are infected. SARS-Cov is completely inactivated by $\leq$ 5 minutes of exposure to 75% ethanol, 500 ppm hypochlorite, and household detergents (25). Disinfection and disinfestations are important in healthcare settings and households. Disinfection of the sewage system, elimination of rodents and cockroaches, and proper garbage disposal are also important. Healthcare workers (HCWs) are engaged in front line to eradicate the SARS. HCWs have a considerable risk to contract the infection if exposed in absence of adapted protection. Rigorous measures of control and educational participations of the population are essential to protect sanitary workers and to prevent the spread of the disease (26).

In the areas of hospitals dedicated to SARS it is mandatory that all HCWs measure their body-temperature twice daily (27). In hospitals not dedicated to SARS, to reduce the contacts between patients and to manage the possible increment of the coming workload from the areas of hospitals dedicated to SARS, surgery and the majority of the out-patients' department's activities would have to be suspended. In order to protect themselves, HCWs must wear N95 masks, gloves and white coats during the contact with any kind of patients. Furthermore, it is likely that the spread of SARS was facilitated by lack of proper hand washing after taking care of SARS patients (28). Compliance monitoring and reinforcement are also needed for maximal effectiveness of infection control measures. Therefore sanitary authorities would have to consider the premature adoption of quarantine procedures. The procedures of isolation and quarantine will be less effective as more cases are introduced. The SARS virus can be transmitted inside of the quarantine areas. Therefore, it is not safe to group suspected cases in isolation rooms. Patients with a diagnosis of suspected SARS are exposed to a high risk to contract the infection if they are grouped with other infected patients (29). These procedures of isolation must be used in combination with developing rapid laboratory assays for SARS. Screening tools using easily available symptomatic and laboratory items are highly desirable. Unrecognized cases of SARS are probably the most important factor that led to intra-hospital spread and cases among HCWs. The non-specific signs and symptoms, long incubation period (mean, 6.4 days), long time between onset of symptoms and hospital admission (from 3–5 days), and lack of a reliable diagnostic test in the early phase of the illness can lead to potential transmission to frontline HCWs and the community. In addition, no pathognomonic signs or symptoms of SARS can be used to differentiate SARS from other causes of community- or hospital-acquired pneumonia. Etiological diagnosis and differentiation from other causes of atypical pneumonia can be made only by laboratory verification. A positive viral culture from respiratory, faecal, and, occasionally, urine or tissue specimens or a four-fold rise in the neutralizing antibody titer in serum samples taken upon admission and 28 days afterward is the most definitive evidence of infection. Among cases of clinical treatments, combinations of steroid either with alfacon-1, a recombinant consensus IFN-α, or protease inhibitors and ribavirin were found to improve outcomes in two different treatment trials using historical controls (30). Due to the very short time course of this epidemic and the initial lack of suitable animal models, randomized control treatment trials are difficult to be organized and executed despite the finding of some commercially available candidate agents that appeared to be active *in vitro*.

Vaccination

For vaccine development, it is critical to generate protective immune response including neutralization antibody and cytotoxic T lymphocytes (CTL) generation. The SARS-Cov is a novel coronavirus, but vaccines for other human coronaviruses have not been successfully developed. Currently, several kinds of SARS vaccines are under development: 1) inactivated SARS-CoV , 2) full-length S protein or N-protein, and 3) those based on fragments containing neutralizing epitopes (31).
Generally, live vaccines are more effective than those using killed microorgnisms but in the case of SARS-Cov, live vaccine is dangerous both for vaccine producer and vaccine receivers. In addition, killed vaccine of SARS-Cov suffers from some additional disadvantages: T-cell independence, major histocompatibility complex restriction and, incomplete virus inactivation may cause SARS outbreaks among the vaccinated population, and some viral proteins may induce harmful immune or inflammatory responses, even causing SARS-like diseases (32). DNA vaccine of spike coding sequence may induce neutralization and specific CTL. Thus, S-based vaccine is considered as a potential candidate. S protein of SARS-CoV, a type I trans-membrane glycoprotein, is responsible for virus binding, fusion, and entry and is a major inducer of neutralizing antibodies (33). S protein consists of a signal peptide and 3 domains: an extracellular domain (13–1193aa), a trans-membrane domain (11194–1215 aa), and an intracellular domain (aa 1216–1255). Its extracellular domain consists of 2 subunits, S1 and S2. Preclinical studies (34) report that a candidate DNA vaccine encoding the full-length S protein induced neutralizing antibodies. Zeng et al. (35) used as DNA vaccine S1 (18-495aa) and S2 gene, rather than the combination with other genes and claimed that S1 and S2 induced high titer of neutralizing antibody. However, the neutralizing antibody was contributed by cooperation of anti-S1 and anti-S2 antibodies (36). Recent studies have demonstrated that a fragment (≈193 aa residues) in the middle of S1 subunit of S protein named RBD, is responsible for virus binding to the receptor on target cells, and induce highly potent neutralizing antibodies against SARS-CoV (36,10).

Several reports have demonstrated that N protein is a representative antigen for the T cell response and may induce cellular and humoral immune responses. N-based vaccines were obtained by use of full nucleocapsid gene including a nuclear location signal with possible pathological risk, thus improving significantly CTL induction.

Discussion

The population-dense regions of Southeast Asia are the epicenter of many emerging diseases, as evidenced by the outbreak of SARS. SARS, the first important infectious disease of 21th century, has taken advantage for a fast international spread without precedence due to the effectiveness of air travels. Moreover, SARS has shown, in a world intimately interconnected and interdependent, the emergence of a new unknown disease that has a remarkably negative impact on the economics, commerce, tourism, and industrial production, as well as on social stability and public health. Rapid identification, epidemiologic surveillance, and prevention of transmission are major challenges in ensuring public health safety (3). Pandemic prevention strategies must be based on preparing for the unexpected and being capable of reacting accordingly. Strategies for survival in the face of emerging pathogens include biotechnology (chemoprophylaxis, vaccines, treatment), public health (for food-borne, water-borne and faecal-borne diseases), and behaviour modification (for sexually transmitted diseases) (37). However, prevention of airborne transmission of microbes still remains a challenge.

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