Characterization of a novel coronavirus responsible for severe acute respiratory syndrome

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Abstract. In February, a new avian H5N1/03 virus took two persons' lives in Hong Kong. After this incidence, a newly emerged disease has been identified, associated with pneumonia in infected patients. Here we report that a newly discovered coronoavirus (Cov) is responsible for this disease. In addition, the basic features, diagnosis and possible animal sources of Severe Acute Respiratory Syndrome (SARS) Cov are also discussed. © 2004 Published by Elsevier B.V.

1. Introduction

Severe Acute Respiratory Syndrome (SARS) is a recently emerged disease associated with pneumonia in a proportion of those human persons infected [1–3]. The outbreak was first recognized in Guangdong Province, China in November 2002 [4]. The disease was unusual for its severity and patients suffering from this disease did not respond to empirical, antimicrobial treatment for acute, community-acquired typical or atypical pneumonia. The clinical syndromes of SARS are fever, shortness of breath, lymphopenia and rapidly progressing changes on radiography. At the end of this outbreak, a cumulative total of more than 8000 cases and 700 deaths have been reported [5]. The disease is highly infectious and >56% of health care workers caring for SARS patients have been infected [6]. A novel coronavirus (Cov) is identified to be the cause of this disease [1,7,8].

2. Identification of SARS Cov at the etiology of SARS

Nasopharyngeal aspirate (NPA) and lung biopsy samples from patients suffering from SARS were used to infect a monkey kidney cell line, FRhK4. Cytopathic effects were observed 2–4 days postinfection. Electron microscopy of negative, stained, infected cells

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showed the presence of pleomorphic, enveloped virus particles of around 80–90 nm (range 70–130 nm) in diameter with surface morphology compatible with a Cov.

In order to elucidate the sequence identity of the virus, total RNA from infected cells was extracted and subjected to random RT-PCR assays [1]. Genetic fingerprints, which were unique in infected cells, were isolated and cloned. Sequence analyses of DNA fragments indicated this virus is close to viruses under the family of Coronaviridae [1]. However, phylogenetic analysis of the protein sequences in this family (types 1–3 Covs) separated the SARS virus into a distinct group [1].

Based on the determined viral sequences, conventional and real-time RT-PCR assays were developed to detect this pathogen in clinical samples [1,9–11]. Besides, a serology test was established to detect IgG antibodies against this virus in patients' sera. More than 93% of SARS patients were reported to be seroconverted at 28 days after the disease onset [1]. By contrast, neither patients with other respiratory diseases nor a normal healthy blood donor had detected antibodies against this virus. Furthermore, it was demonstrated that this newly discovered virus could cause similar clinical presentation in cynomolgus macaques (*Macaca fascicularis*) [12,13]. Thus, the overall results fulfilled Koch's postulations that the etiological agent of SARS is the SARS Cov.

3. SARS diagnosis

Because SARS is a highly contagious disease, a rapid identification of SARS patients would not only allow prompt clinical treatments and patient management, but also establishing a quarantine policy, thereby minimizing the risk of cross-infection [14]. The identification of SARS Cov prompted us to develop tests for SARS diagnosis. The first approach depends on the detection of antibodies against SARS Cov from SARS patients [1]. This kind of serological assay is highly accurate [15] and IgG seroconversion is reported to start at day 11 of disease onset [15]. Thus, serological tests might be useful for confirmatory purposes.

The second test for SARS diagnosis is based on detecting the SARS Cov viral RNA. Detection of the virus by RT-PCR in clinical specimens offers the option of diagnosis in the early stage of the disease. In this outbreak, we developed several conventional and real-time quantitative PCR assays for SARS diagnosis [1,9–11]. Quantitative analysis of viral RNA in NPA specimens indicated that the viral loads at the early disease onset are low and peak at day 10 of the illness [15]. The presence of low copy numbers of a viral genome in early clinical samples is a major limiting factor for achieving a sensitive molecular test for SARS diagnosis. Recently, we demonstrated that this problem could be overcome by increasing the volume of clinical samples for RNA extraction. By using a modified RNA extraction method and applying quantitative real-time RT-PCR technologies, 80% of specimens collected at days 1–3 of the onset were positive in RT-PCR assays [10].

4. SARS Cov is of animal origin

To better understand the animal hosts involved in the ecology and interspecies transmission of this virus, an investigation was carried out in a retail live animal market in Guangdong, mainland China [16]. SARS Covs were isolated from four out of six

Himalayan palm civets (*Paguma larvata*, Family Viverridae). Evidence of virus infection was also detected in a raccoon dog (*Nyctereutes procyonoides*), Chinese ferret badger (*Melogale moschata*) and in humans working in direct contact with these animals [16]. Phylogenetic analysis indicates that these animal viruses are highly similar to human SARS Cov [16]. Sequence analysis revealed that all the animal virus isolates retain an ''additional'' 29 nucleotide sequence, not found in most human virus isolates, which results in encoding a new putative protein of 122 amino acids of unknown function.

5. Conclusion

Both H5N1 influenza and SARS emerged in the hypothetical pandemic influenza epicenter of southern China. In the case of H5N1, chickens were the immediate source of virus for humans [17]. The detection of SARS Cov-like viruses in small wild mammals found in live retail markets supplying the restaurant trade in Guangdong indicates how this virus may have crossed from its animal reservoir to humans. Surveillance of SARS Cov in animals is important for public health and may provide clues to understanding the interspecies transmission events relevant to the genesis of novel, emerging diseases.

Acknowledgements

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