Short communication

Probing the structure of the SARS coronavirus using scanning electron microscopy

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A novel coronavirus, SARS-CoV, has been confirmed to be the aetiological agent of SARS. Transmission electron microscope (TEM) images played an important role in initial identification of the pathogen. In order to obtain greater morphological detail of SARS-CoV than could be obtained by TEM, we used ultra-high resolution scanning electron microscopy (SEM) to image the virus particles. We show here the three-dimensional appearance of SARS-CoV. Enhanced detail of the ultrastructure reveals the trimeric structure of the 10–20 nm spikes on the virion surface. These results contribute to characterization of the SARS agent and development of new antiviral strategies.

Introduction

It has been confirmed by several research groups [1–4] that the worldwide outbreak of severe acute respiratory syndrome (SARS) is caused by a novel coronavirus, named SARS coronavirus (SARS-CoV). The ultrastructural features of coronaviruses, including SARS-CoV, have been established by transmission electron microscope (TEM) imaging [2]. However, little is known about the appearance of a complete virus particle and the ultrastructure of the virus surface. Here we provide the first observation of SARS-CoV particles by means of a dedicated ultra-high resolution scanning electron microscope (SEM) (HITACHI S-5200, Japan). These data contribute to a more comprehensive understanding of SARS-CoV.

Materials and methods

Preparation of purified SARS virus

Given the serious nature of SARS, all clinical specimens were handled in a biosafety level 3 laboratory. Serum specimens were inactivated by β-lactone before outside serological testing. SARS virus strain BJ01 [5] was obtained from a patient with SARS and established in the Vero E6 cell line. Culture supernatant was collected and centrifuged at low-speed to remove cells fragments. After sucrose-gradient ultracentrifugation, the purified virus pellet was resuspended in TNE (0.25 M NaCl, 0.02 M Tris-Cl pH 7.5, 0.001 M EDTA) buffer.

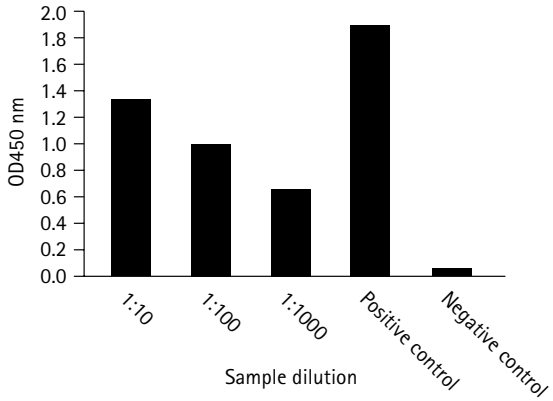
Enzyme-linked immunosorbent assay

Inactivated virions were coated onto 96-well plates and incubated with either normal sera or convalescent sera from SARS patients, and then detected by HRP conjugated anti-human IgG. Positive and negative controls were from a diagnostic kit of antibodies for SARS virus (BGI-GBI Biotechnology Ltd, Beijing, China) and were independent of the sera we used. The cut off value was determined by the equation: cut off value=0.13+$\text{Value}_{\text{negative control}}$ (OD450 nm). If the colour reaction represented a higher value than the cut off value, it was judged as positive.

Sample preparation for scanning electron microscopy

The purified viruses were submitted to the fixation and sputter coating procedure before viewing by SEM. Briefly, 3×5 mm cover slides or 200 mesh copper grids were coated with 1% poly-L-lysine (87000) and then rinse with phosphate-buffered saline (PBS, pH 7.2) to remove excessive poly-lysine. Viruses with different dilution in TNE were dripped on cover slides or copper grids and let adhere for about 30 min. Excessive samples were sucked up and rinsed with PBS. After fixation in 2.5% glutaraldehyde for 30 min and a further 30 min in 1% osmium tetroxide, samples were dehydrated through an ethanol series in buffer: 50% – 70% – 90% – 100% – 100% for 5 min each and critically point dried from ethanol. Samples were mounted

Figure 1. Specific binding of the purified SARS-CoV BJ01 to convalescent sera from SARS patients



on specimen stubs with conductive paint and coated to a thickness of 10 nm with Au in a sputter coater. Samples were viewed in lens on the Hitachi S-5200 SEM with slow scan mode, 10 kV accelerating voltage and 0–0.3mm working distance.

Results

In order to identify the biological characteristics of the purified virus, we first tested the reactivity of the virus with convalescent sera from SARS patients using an enzyme-linked immunosorbent assay (ELISA). Comparing with positive and negative controls, the purified SARS-CoV showed very strong binding to the convalescent sera but not to normal sera (Figure 1). This specific reaction indicated that the virus we purified from the culture supernatant of Vero E6 cells was SARS-CoV.

To better understand the morphological detail of SARS-CoV, we used ultra-high resolution scanning electron microscopy to image the virus particles. Under the SEM, we observed large numbers of virus particles with predominantly 150–200 nm in diameter either isolated or in aggregates (Figure 2). These SARS-CoV particles with a typical crown structure were further confirmed by using TEM (data not shown). Incidentally, we found a few virus particles as large as 400 nm in diameter, which had identical shape and surface substructure as the 200 nm particles and presented in a single field (Figure 2C).

The virus particles appear round and full, with numerous tiny surface projections. Some are tightly adhered with their projections sticking into each other, forming a mosaic patch and leading to the compression of the virions. These flower-shaped projections corresponding to the spike, the main constituent of which was S protein, have a size ranging from 10 to 20 nm, in agreement with the reported diameter of the SARS virus spike. With further magnification of this part (Figure 2D, arrowheads), we observed the projections with a regular structure composed of three subunits, like a flower with three petals.

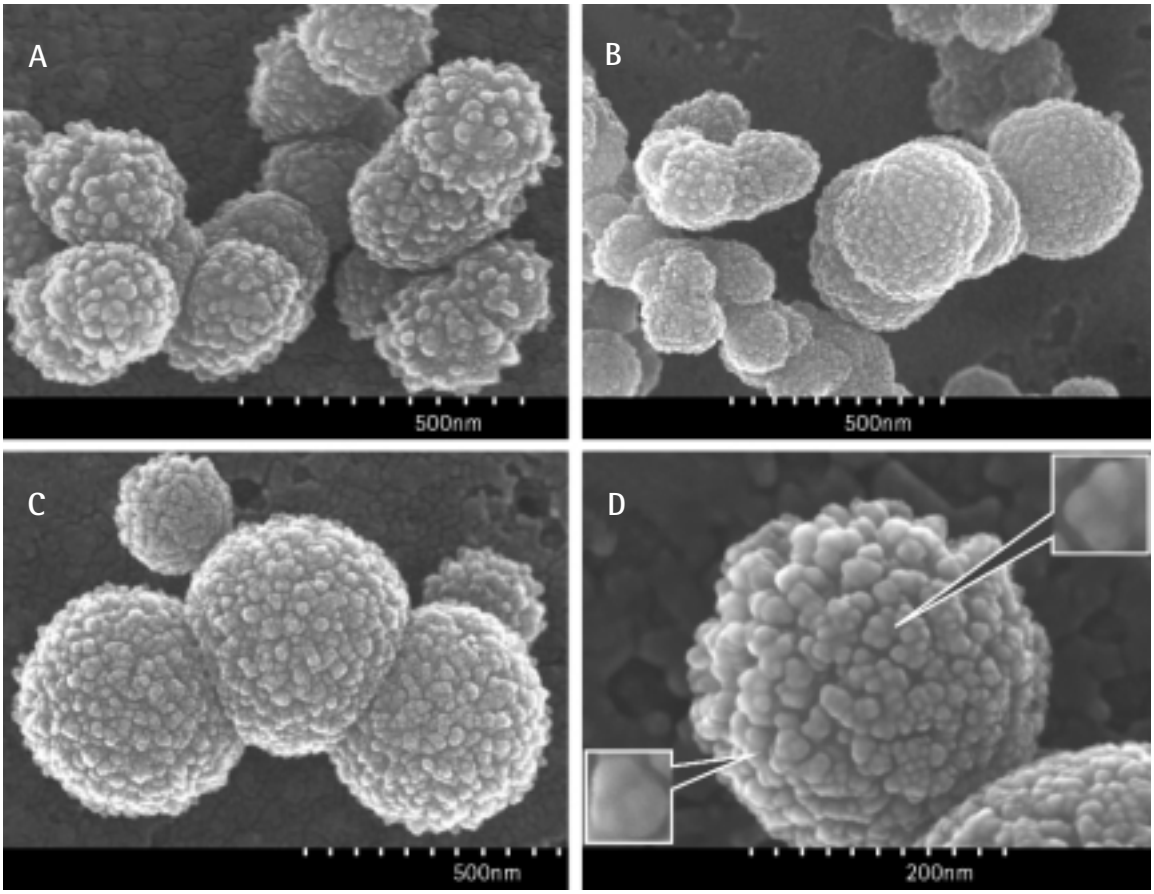
Conclusion

In this study, we provide the first three-dimensional structure of the causative agent in SARS infection and the detailed information about the ultrastructural surface of SARS-CoV by using SEM, which offers advantages over TEM for the study of biological molecules such as viruses, nucleic acid and proteins, due to its higher resolution and less disruptive nature of the technique. We observed all the surfaces of SARS virions bear flower-shaped projections that contain three S protein subunits. The S glycoprotein is known as a major immunogenic determinant of pathogenesis, binds to specific receptors and then undergo a temperature-dependent, receptor-mediated conformational change that leads to fusion of the viral envelope with host membranes to initiate infection. Delmas *et al*. demonstrated by *in vitro* experiment that coronavirus S protein tends to oligomerize into a trimer and the trimerization was a rate-limiting step after polypeptide synthesis [6]. Together with our structural observations of trimeric S protein on the surface of virion, we hypothesize that the identification of the receptor binding sites and the conformational change after S protein activation, as well as molecular interpretation of trimerization, may give rise to new strategies for development of novel antiviral drugs.

It has been accepted that the size of coronaviruses are about 80–200 nm observed by TEM. However, we found in the SEM images that the size of the SARS CoV BJ01 varied, most of them were about 150–200 nm, a few up to 400 nm in diameter, which is significantly larger than the known 80–220 nm size of SARS-CoV, even if the thickness of Au coating (20 nm) was added. We noticed that the larger virions had the same flower-shaped projections as those with normal size, and both particles in different sizes were seen in the same view. Although we are not able to prove these variations by further experiments at the moment because of the lack of SARS-CoV material, it remains an interesting question to be addressed in future.

Taken together, our data vividly revealed the three-dimensional appearance of SARS-CoV as well as their ultrastructural surfaces with three subunits-based projections. These results would enrich the morphological studies of the SARS-CoV and contribute to better understanding of SARS-CoV, with application to both basic virology and clinical practice.

Figure 2. SARS-CoV observed under scanning electron microscope



(**A**) Virions with diameter of 200 nm. (**B**) Virions with sizes of 100 and 200 nm. (**C**) Virions of 400 nm in diameter. (**D**) The ultrastructure of the surface projections. Two typical spikes are magnified to show the trimer structure (insets).

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