Demonstration of 7-nm Projections on Human and Avian Coronaviruses

Elisabeth Kjeldsberg, MSc

Department of Virology, National Institute of Public Health, Oslo, Norway

Direct electron microscopy (EM) of human coronavirus (HCV) strain OC43 and avian infectious bronchitis virus (IBV) strain F revealed particles with small granular projections about 7 nm in size in addition to the characteristic coronavirus particles with projections of 20 nm. Relationship of the short fringed form to the conventional coronavirus particle is established by ordinary immune electron microscopy (IEM) and immunosorbent electron microscopy (ISEM).

KEY WORDS: coronavirus, granular projections, infectious bronchitis virus, electron microscopy, immune electron microscopy, immunosorbent electron microscopy.

INTRODUCTION

The coronoviruses constitute a morphologically distinct group of viruses that infect a number of animal species and humans.[1] Berry et al.[2] were the first to observe the bulbous projections of infectious bronchitis virus (IBV), and a few years later Almeida and Tyrrell[3] described clearly the distinct morphology of human coronavirus (HCV) and its resemblance to IBV. Since then several reports on the morphology of coronaviruses have been published.

Coronaviruses are pleomorphic, roughly spherical particles with a characteristic corona of widely spaced surface projections that show an extremely narrow stalk and dilations at the distal end. Different coronaviruses vary in size of the total diameter, which ranges from 60–220 nm in negatively stained preparations.[1] Furthermore, the surface projections vary in size, with lengths between 12 and 24 nm, and in shape of the dilations.[4] In addition to the characteristic solar corona, a shorter fringe, 5–10 nm in length, consisting of small granular projections has been demonstrated on enteric coronaviruses from calves[5] and infant mice[6] and on a candidate strain of human enteric coronavirus.[7,8] The present report presents similar findings by electron microscopy of respiratory HCV and IBV.

MATERIALS AND METHODS

HCV strain OC43 was propagated in Vero cell monolayers. IBV strain F, antigenically closely related to the Massachusetts strain[9] and passaged 39 times in embryonated eggs, was kindly supplied by Dr. J. Krogsrud, Veterinary Institute, Oslo. HCV in cell cultures and IBV in allantoic fluid were kept frozen at −20°C until use. For direct electron microscopy (EM) the virus suspensions were gently thawed and clarified by low speed centrifugation for 5 min at 2000 g and the supernatants recentrifuged for 1 h at 35,000 g. The pellets were suspended in distilled water, negatively stained with 2% w/v phosphotungstic acid (PTA), pH 6.5, and examined in a JEM 100B electron microscope at a magnification of 50,000X, which was calibrated with a diffraction grating specimen.

All sera used in immune electron microscopy (IEM) and immunosorbent electron microscopy (ISEM) were heat inactivated for 30 min at 56°C and ultracentrifuged for 1 h at 100,000 g before use. IEM was performed with rabbit antiserum and human convalescent serum with high titers in enzyme-linked immunosorbent assay (ELISA) against HCV strain OC43 (a gift

The author thanks Professor M. Degré for reviewing the manuscript and Dr. J. Krogsrud and Dr. I. Ørstavik for supplying the infectious bronchitis virus and the antiserum to HVC strain OC43, respectively.

from Dr. I. Ørstavik, Ullevål Hospital, Oslo). A 0.4-ml aliquot of virus suspension was incubated at 4°C overnight with 0.1 ml of a 1/10 dilution of serum in phosphate-buffered saline (PBS). The incubation mixture was centrifuged for 1 h at 15,000 g and the pellet negatively stained with PTA. ISEM was carried out as earlier described.[10] Grids were coated with protein A (Pharmacia), 10 μg/ml, followed by normal rabbit serum or rabbit antiserum against HCV strain OC43, both diluted in PBS. The coated grids were incubated overnight at 4°C with virus suspension. After careful washing in distilled water the grids were negatively stained and examined in the electron microscope as described above.

RESULTS

Electron Microscopy

Electron microscopic examination of the HCV strain OC43 showed particles with varying morphology (Fig. 1). Typical coronavirus particles with large surface projections were observed (Fig. 1*a*). The projections were widely spaced on the envelope. They were approximately 20 nm long with a rather thin stalk widening at the distal end. The envelope seemed to be intact. Other morphologic types of viruslike particles (Fig. 1*b*) were of about the same size as the typical coronaviruses and occasionally showed a central electron-dense area. Some of the particles were covered with a short fringe of projections while others showed only a few or no projections at all. The small granular projections measured approximately 7 nm and were closely spaced on the envelope (Fig. 1*c*). A suggestion of two layers of projections was seen on a few particles (Fig. 1*d*).

IBV showed the same variation in morphology as described for HCV. Particles with 20-nm projections (Fig. 2*a*) and with 7-nm projections (Fig. 2*b*) and particles without projections (Fig. 2*c*) were all demonstrated. In this case,

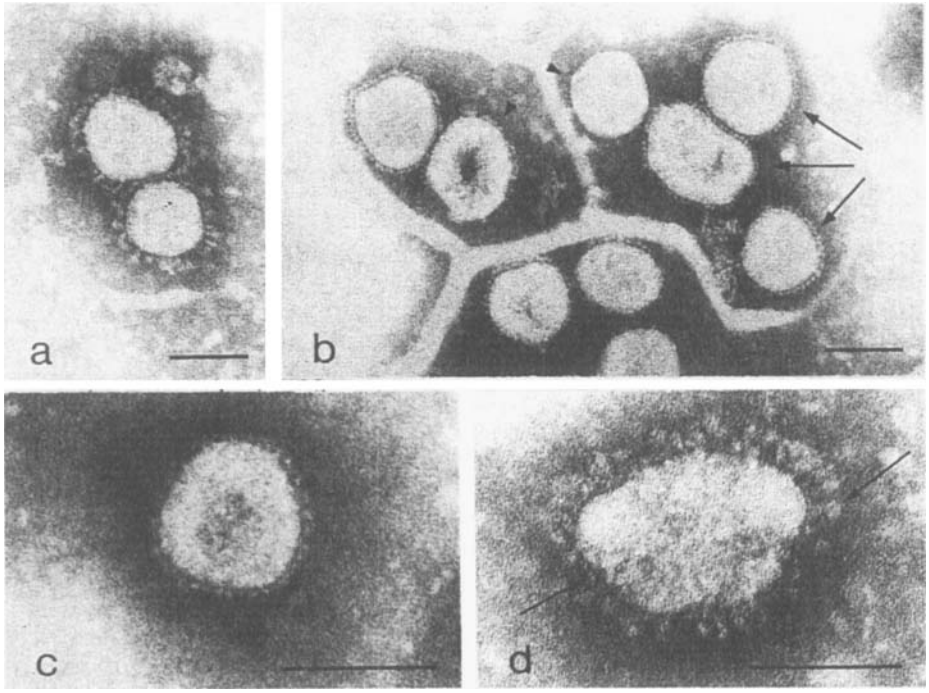

FIG. 1 *HVC strain OC43 from Vero cell culture. Negatively stained with 2% phosphotungstic acid, pH 6.5. Bars represent 100 nm.* (a) *Typical coronaviruses with 20-nm projections.* (b) *Viruslike particles with short 7-nm projections* (arrows) *and particles with few or no projections at all* (arrowheads). (c) *Higher magnification of particle with 7-nm projections.* (d) *Higher magnification of particle with a suggestion of two layers of projections* (arrows).

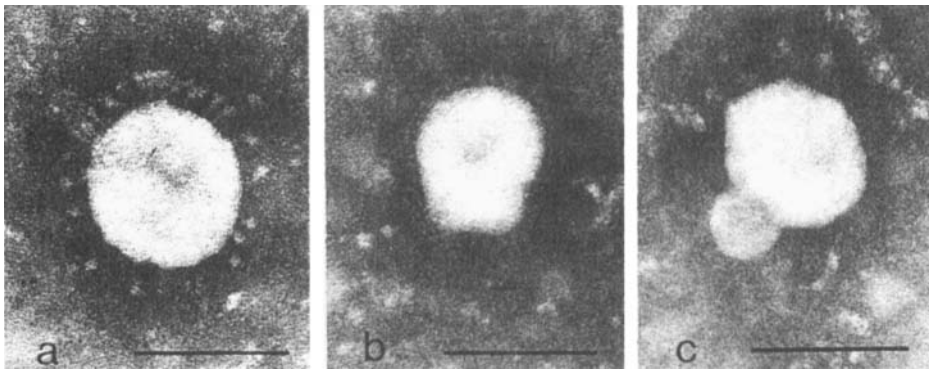

FIG. 2 *Avian infectious bronchitis virus from allantoic fluid of infected eggs. Negative staining with 2% phosphotungstic acid, pH 6.5. Bars represent 100 nm.* (a) *Typical IBV particle with wide-spaced, club-shaped 20-nm projections.* (b) *Viruslike particle with short, dense fringe of 7-nm projections.* (c) *Virus particle without projections but showing extrusion typical of coronavirus.*

however, large and small projections were not seen on one and the same particle.

None of the above mentioned morphologic types of virus particles were seen in uninfected Vero cells or allantoic fluid.

Immune Electron Microscopy

By ordinary IEM of HCV strain OC43 large aggregates were formed with rabbit antiserum as well as with human convalescent serum. Figure 3 shows co-aggregation between all three morphologic forms and rabbit serum. The majority of the particles in the aggregate lacked projections, but some were short fringed forms and a few showed long projections on part of their surface. Antibody molecules were seen on and between the particles. No aggregates occurred when normal sera were used.

Immunosorbent Electron Microscopy

By ISEM of HCV strain OC43, a considerably higher number of viruslike particles with structures as illustrated in Figure 1*a–c* were adsorbed to grids coated with specific antiserum against HCV strain OC43 than to grids treated with normal rabbit serum (Table 1). The number of particles adsorbed decreased with higher dilutions of the antiserum.

COMMENT

The co-aggregation by conventional IEM and the specific adsorption to antibody-coated grids by ISEM strongly suggest all three morphologic forms to be coronaviruses.

Short projections have so far been demonstrated mainly on virus particles grown in vitro. The role of these structures as the basal part of the large projections has been proposed by others.[6] The basal part of the large projections however shows a narrower diameter than seen on the small projections, and often the basal part is not visible at all, which gives the impression of the solar corona. It seems more likely that they have other functions, and results obtained in examination of a coronavirus associated with diarrhea in infant mice strongly suggest the hemagglutinating activity (HA) to be related to the small granular structures.[6] The HA of IBV has been found not to be associated with intact infectious virus[11] and could be related to the short projections demonstrated in this note. Further work needs to be done on IBV and HCV to clarify this relationship.

Particles similar to those described in this paper are seen in clinical specimens, especially fecal samples, from time to time but have been neglected due to lack of knowledge of their identity. In viral diagnosis it has been a problem to identify coronaviruses by electron microscopy due to the pleomorphic structure and instability of the characteristic projections, and a lot of coronaviruses have probably

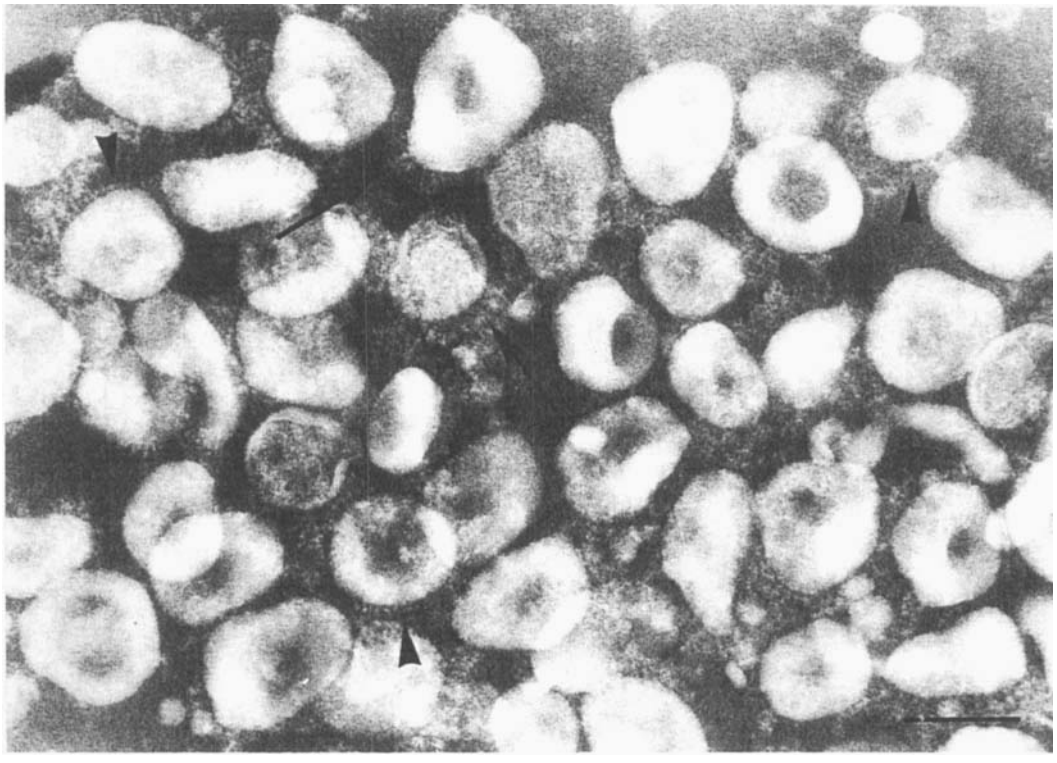

FIG. 3 *Immune electron microscopy with HCV strain OC43 and specific rabbit antiserum. Negative staining with 2% phosphotungstic acid, pH 6.5. Bar represents 100 nm. Co-aggregation between long* (arrows) *and short* (arrowheads) *fringed forms and particles without projections.*

been overlooked by EM examination. More information on the varying morphologic appearances of coronavirus would be a help in diagnostic work.

TABLE 1 Adsorption of Coronaviruses from a Freeze-Thawed HCV Strain OC43 Infected Vero Cell Suspension: Immunosorbent Electron Microscopy Using Rabbit Sera

Serum dilution	No. of particles*	
	Normal serum	Antiserum
1/10	4	115
1/100	3	70
1/500	3	43

*Total count on five 60 X 90 mm micrographs at a magnification of 10,000X.

REFERENCES

1. McIntoch K: Coronaviruses: A comparative review. Curr Top Microbiol Immunol 63:85–129, 1974.
2. Berry DM, Cruickshank JG, Chu HP, Wells RJH: The structure of infectious bronchitis virus. Virology 23:403–407, 1964.
3. Almeida JD, Tyrrell DAJ: The morphology of three previously uncharacterized human respiratory viruses that grow in organ culture. J Gen Virol 1:175–178, 1967.
4. Davies HA, Macnaughton MR: Comparison of the morphology of three coronaviruses. Arch Virol 59:25–33, 1979.
5. Bridger JC, Caul EO, Egglestone SJ: Replication of an enteric bovine coronavirus in intestinal organ cultures. Arch Virol 57:43–51, 1978.
6. Sugiyama K, Amano Y: Morphological and bio-

logical properties of a new coronavirus associated with diarrhea in infant mice. Arch Virol 67:241–251, 1981.
7. Macnaughton MR, Davies HA: Human enteric coronaviruses. Arch Virol 70:301–313, 1981.
8. Patel JR, Davies HA, Edington N, Laporte J, Macnaughton MR: Infection of a calf with the enteric coronavirus strain Paris. Arch Virol 73:319–327, 1982.
9. Estola T: Studies on the infectious bronchitis virus of chickens isolated in Finland. Acta Vet Scand, Suppl 18, pp. 1–111, 1966.
10. Kjeldsberg E, Mortensson-Egnund K: Comparison of solid phase immune electron microscopy, direct electron microscopy and enzyme-linked immunosorbent assay for detection of rotavirus in faecal samples. J Virol Methods 4:45–53, 1982.
11. Biswal N, Nazerian K, Cunningham CH: A hemagglutinating fraction of infectious bronchitis virus. Am J Vet Res 27:1157–1167, 1966.

Accepted in revised form 20 June 1984.