Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Kidney International (2020) **98,** 228–231; https://doi.org/10.1016/j.kint.2020.04.006

Visualization of putative coronavirus in kidney

To the editors: We read with concern the articles that report the presence of coronavirus in kidney based on electron microscopic evidence.[1,2] Neither article, in fact, demonstrates the presence of coronavirus in the kidney. Su *et al.*[1] show purported virus particles in the cytoplasm of kidney tubular epithelium and podocytes. These structures are not viral particles, but rather clathrin-coated vesicles, normal cell organelles involved in intracellular transport. The objects in their Figure 2a and b (∼60 nm) are somewhat smaller than coronaviruses (∼80 to 140+ nm), but more importantly, their "spikes" (peplomers) are in contact with the cytosol, as are those on clathrin-coated vesicles; the larger particle in Figure 2d also has spikes that are touching the cytosol and does not have dense dots inside the particles corresponding to the coiled nucleocapsid, cut in cross section. Coronaviruses, on the other hand, have their projections either facing the extracellular space between cells or the space inside vacuoles within the cells.[3–5] This phenomenon is due to the fact that coronaviruses receive their outer covering by budding into or on cellular membranes, thereby forming intracellular vacuoles with the viral projections in contact with the vacuolar content, not the cytosol. During assembly, viral structural proteins are incorporated into the endoplasmic reticulum–Golgi complex of the infected cell, and viral RNA, packaged with another protein, buds into these membranes, forming a membrane-bound sac containing mature virions; the spikes are on the outside of the virion, but inside the vacuole and not in direct contact with the cytosol (Figure 1). These virions get out of the cell by exocytosis when the vacuole membrane fuses with the plasma membrane and opens its contents to the outside; thus, complete virions with peplomers are seen within the cell inside the membrane container (sequestered from the cytosol) and outside of cells, frequently still attached to the opened vacuolar membrane that has fused with the plasma membrane. The particles shown in electron micrographs in the article by Su *et al.*[1] have their spikes in contact with the cytoplasmic fluid, like endocytotic vesicles, that is, clathrin-coated vesicles (see Plate 523, Figures 3–5, pp. 1214–1215 in Ghadially[6]; Figure 18c and d in Miller[7]; and Miller[8]).

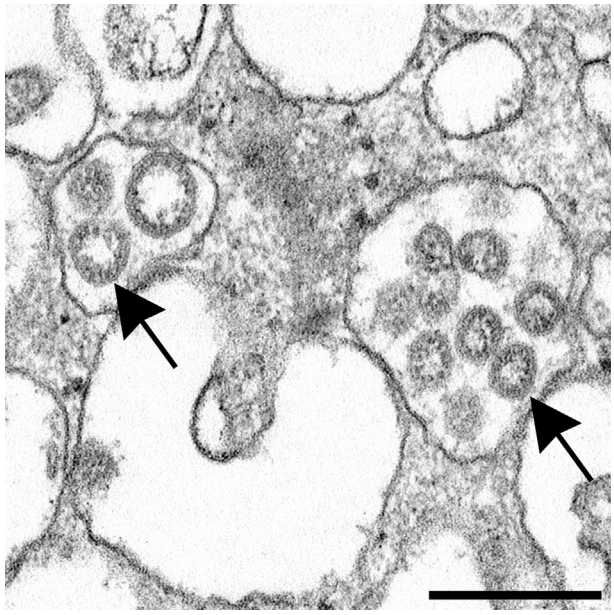

Figure 1 | Electron microscopic image of an isolate of severe acute respiratory syndrome coronavirus 2 seen here inside vacuoles (arrows). Note the dense membrane coat around the viral particles. This micrograph is of viral particles in a cell culture inoculated with infected patient nasopharyngeal and oropharyngeal fluids. Bar = 200 nm. Image provided by Cynthia S. Goldsmith, Centers for Disease Control and Prevention. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

Likewise, the particles in Kissling *et al.*[2] are not coronaviruses. While they are inside a vacuole, have spikes, and are approximately the correct size, they do not have the uniform appearance of virus particles with a membrane outer covering and dots inside indicating the nucleocapsid.[3–5] These objects are inside a vesicle called a multivesicular body (see Plates 277–278, pp. 632–634 in Ghadially[6]; Calomeni *et al.*[9]; and Figure 3, p. 393 in Haguenau[10]). The article by Kissling *et al.*[2] is concerning, as electron microscopy is the only alleged evidence presented in support of the suggestion that coronaviruses are actually present in this kidney tissue; all other tests for coronavirus in kidney were negative. These micrographs do not support the statement that the particles are indeed viruses.

Knowledge of virus morphology and morphogenesis, as well as of cellular architecture, is necessary to distinguish viral pathogens from normal subcellular organelles. This distinction is frequently difficult, because numerous cellular components can masquerade as viruses.[7–11]

ACKNOWLEDGMENTS

We are grateful to Cynthia S. Goldsmith, Centers for Disease Control and Prevention, for critically reviewing this letter, providing helpful suggestions, and supplying the figure of severe acute respiratory syndrome coronavirus 2. We also thank Dr. David N. Howell for reviewing this manuscript.

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Kidney International (2020) **98,** 231–232; https://doi.org/10.1016/j.kint.2020.05.004

The authors reply: We thank Drs. Miller and Brealey[1] for their comments and fully acknowledge their expertise in the field of electron microscopy. We also acknowledge our uncertainties regarding the exact nature of the particles seen in the podocytes in our patient's kidney biopsy, and we were cautious in the interpretation of these findings. Following Drs. Miller and Brealey's comments,[1] we have modified our letter before its final publication in the journal to further underline that these particles may correspond to nonviral entities.

However, the particles detected in our patient's biopsy are rather similar to the ones reported in the first documentation of severe acute respiratory syndrome coronavirus 2.[2] Besides, the appearance of intracellular viral inclusions appears to be quite variable from one publication to another.[3,4] To our opinion, it remains, therefore, possible that the particles observed in our patient are of viral origin. Nevertheless, we totally agree with Drs. Miller and Brealey[1] that the definite proof for the presence of viral inclusions in cells requires an immunostaining with specific antibodies, whether in cultured cells or in tissue samples.

Our knowledge of coronavirus disease 2019 is rapidly evolving and caution is of the utmost importance.

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Kidney International (2020) **98,** 232; https://doi.org/10.1016/j.kint.2020.05.002

The authors reply: We have carefully read and considered the letter from Prof. Miller and Dr. Brealey,[1] distinguished experts of electron microscopy (EM), and appreciate that they pointed out the limitations of our study.[2]

We agree with Miller and Brealey's point and recognize that there are inherent difficulties in discrimination of cellular vesicles from viral particles solely by morphological evidence, especially in routine EM processing of autopsy tissues. These conditions differ markedly from the *in vitro* negative staining of body fluids or cell culture, which are the techniques usually utilized for optimal visualization of viral structure. However, EM is still an essential tool and a front-line evaluation method in the search for unknown pathogens in outbreaks or epidemics. For example, the causative agents of the outbreak of severe acute respiratory syndrome (SARS) in China in 2003 and human monkey pox in the United States in 2003 were both first identified by EM. In addition, with our immunofluorescence staining for SARS-coronavirus (CoV) nuclear protein as we presented in our paper (Figure 3d)[2] and the recent publications of ultrastructural feature of SARS-CoV-2,[3,4] we consider the structures as possible, but not definitively proven, CoV. We have therefore prudently changed the description in the preprint version of our article of "viral particle" to "coronavirus-like particle." Ideally, immuno-EM or *in situ* hybridization studies to assess local protein or RNA levels of CoV will further clarify the possibility of direct kidney parenchymal infection. Such a combination of ultrastructural images and molecular data could then definitively identify viral-like particles as SARS-CoV-2.

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