SARS-CoV-2 cytopathogenesis in cultured cells and in COVID-19 autptic lung, evidences of lipid involvement.

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Keywords: SARS-CoV-2, COVID-19, SARS-CoV-1, Electron Microscopy, Lung, Lipid droplets, Cell death

Posted Date: July 7th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-39274/v1>

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Abstract

Background: The pathogenesis of SARS-CoV-2 remains to be defined. Elucidating SARS-CoV-2 cellular localization within cells and its cytopathic effects requires definition. We performed a comparative ultrastructural study of SARS-CoV-2 infection of Vero-6 cells and lung from COVID-19 patients.

Main findings: SARS-CoV-2 induces rapid ultrastructural changes and death in Vero cells. Ultrastructural changes in SARS-CoV-2 infection differ from those in SARS-CoV-1. Type II pneumocytes in lung tissue showed prominent altered morphological features with numerous vacuoles and swollen mitochondria with presence of abundant lipid droplets. The accumulation of lipid droplets was the most striking finding we observed in cultured cells and in infected pneumocytes. Virus particles were also found associated with lipo-lysosomes suggesting that they can play an important step in virus assembly.

Interpretation: The cytopathology of SARS-CoV-2 appears to be different to that caused by SARS-CoV-1. Our findings highlight important open topics which may represent future targets to contrast the pathogenicity of SARS-CoV-2.

Introduction

Since the first discovery of SARS-CoV-2 as a novel human zoonotic pathogen in late December, 2019 [Ref 1], there have been 357, 688 deaths from COVID-19 disease reported to the WHO of May 30th, 2020 [Ref 2]. The pathogenesis of SARS-CoV-2 remains to be defined. Current knowledge of COVID-19 pathogenesis is evolving, and is based on specific SARS-CoV *in vitro* studies and from autopsy studies, and extrapolations from what is known from two other zoonotic coronaviruses which have jumped the species barrier to humans, SARS-CoV and MERS-CoV [Refs 3–8].

Various mechanisms for tissue pathology in COVID-19 have been proposed, including direct cytopathic effects, ischemic injury and immune-pathology due to excessive and aberrant immune responses. SARS-CoV-2 infects the host cells using the angiotensin converting enzyme 2 (ACE2) receptor [Ref 9], which is expressed in cells and vessels of several organs, including the lung, heart, kidney, intestine. Coronaviruses have a lipid bilayer derived from the host cell membranes and thus the intracellular membrane may play a key role in replication and cytopathic effects.

Evaluation and determination of SARS-CoV-2 virus distribution within tissues, cellular localization and its cytopathic effects is important for elucidating the pathogenetic mechanisms of SARS-CoV-2. Ultrastructural studies carried out so far, didn't show clear but rather conflicting findings concerning the presence of viral particles inside different tissues [Refs 10–12].

In vitro cytopathic studies of SARS-CoV-2 using cell lines may not capture *in vivo* pathology in affected body organs and thus performing studies in parallel is important. We thus performed a comparative

ultrastructural study of SARS-CoV-2 infection of Vero-6 cells and lung tissue cells from 20 patients who died of COVID-19 disease. We also investigated the effects of SARS-CoV-2 on Vero cells, compared to effects of SARS-CoV-1.

Methods

SARS-CoV-2 and SARS-CoV-1 isolates

SARS-CoV-2: The first COVID-19 cases were identified on January 31st at our National Institute for Infectious Diseases IRCCS “Lazzaro Spallanzani”, Rome, Italy. SARS-CoV-2 was isolated and cultured from these patients [13] and was used in this study.

SARS-CoV-1: SARS-CoV-1 (Tor2 isolate kindly provided by National Microbiology Laboratory, Public Health Agency of Canada).

VERO cell lines and infection with SARS-CoV-2 and SARS-CoV-1

Mammalian cell lines Vero E6 were cultured in Dulbecco’s essential medium (DMEM, Sigma-Aldrich) containing 10% fetal bovine serum (FBS), at 37°C in a 5% CO₂ atmosphere. Sub-confluent cells were infected with the SARS-CoV-2 INMI1 isolate (named 2019-nCoV/Italy-INMI1, GISAID accession number: EPI_ISL_410546) obtained from sputum sample (mucus and phlegm coughed-up from the lower airways) collected at admission from the first Covid-19 patient reported in Italy in January 2019 and hospitalized at INMI.

Specifically, the cells were exposed to the SARS-CoV-2 (2019-nCoV/Italy-INMI1) isolate in DMEM not containing FBS for 1 hour at 37°C; at the end of the adsorption period, cells were washed, and maintained in DMEM plus 2% FBS. The same method was used for infection of Vero cells with SARS-CoV-1. Uninfected Vero cells were used as controls.

Negative Staining

Purified SARS-CoV-2 and SARS-CoV-1 viral suspensions were fixed in 2.5% glutaraldehyde and allowed to adsorb onto a formvar carbon-coated grid for a few minutes before being stained with 1% phosphotungstic acid for 1 min. The excess fluid was blotted and the grid left to dry before viewing under a transmission electron microscope JEOL JEM 2100 Plus (Japan Electron Optics Laboratory Co. Ltd. Tokyo). Images were captured digitally with a digital camera TVIPS (Tietz Video and Image Processing Systems GmbH, Gauting, Germany).

Autopsy Lung tissues

Post-mortem examination of 20 consecutive patients, who died of laboratory confirmed COVID–19 disease, were performed at the National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS Hospital (Rome, Italy). Autopsies were performed according to guidance for post-mortem collection and submission of specimens and biosafety practices (CDC March 2020, Interim Guidance; Hanley B, Lucas SB, Youd E, Swift B, Osborn M. Autopsy in suspected COVID–19 cases. *J Clin Pathol.* 2020;73(5):239-242. doi:10.1136/jclinpath–2020–206522) to reduce the risk of transmission of infectious pathogens during and after the post-mortem examination. The study was approved by the local Clinical Research Ethics Committee (approval number: n° 9/2020).

Specimens from lungs were fixed in 10% neutral-buffered formalin, and routinely processed to paraffin blocks. Sections of tissues (4 µm) were stained with hematoxylin and eosin (H&E). For immunohistochemistry deparaffinized and rehydrated sections were used. Immunostaining was performed on BenchMark ULTRA system fully automated instrument (Roche) with antibody directed against CD68 Ventana (KP–1), and anti-Coronavirus (FIPV3, Santa Cruz).

Light and Transmission electron microscopy

Light and transmission electron microscopy (TEM) was performed on Vero cells and autopsy lung tissue specimens using standard procedures. Cultured cells and small pieces of tissues were fixed with 2.5% glutaraldehyde in 0.1M cacodylate buffer, for 4h at 4 °C. Post-fixation was performed with 1% OsO₄. Samples were then dehydrated in graded ethanol and embedded in Epon resin, as previously described [14,15]. Ultrathin sections were stained with 2% uranyl acetate and observed under a transmission electron microscope JEOL JEM 2100 Plus (Japan Electron Optics Laboratory Co. Ltd. Tokyo, Japan). Images were captured digitally with a digital camera TVIPS (Tietz Video and Image Processing Systems GmbH. Gauting, Germany).

Results

Negative Staining

Negative staining electron microscopy of purified SARS-CoV–2 particles revealed a spherical (*Fig. 1A*) or slightly pleomorphic shapes (*Fig. S1A,C,D*).. On the surface of the virions the typical rim of cone-shaped spikes were identified, but their distribution was not as regular as usually reported for other Coronavirus, in fact they appeared in multi-aggregated fashion (*Fig. 1A, Fig. S1*).. The diameter of the viruses ranged from 80 to 102 nm (average size 93.61 nm), while the length of the spikes ranged from 9 to 12.5 nm (average length 10.99 nm). Some of the viral particles showed part of the ribonucleic-protein material extruding from rupture of the envelope (*Fig. S1C*)..

EM findings - SARS-CoV–2 infected Vero cells.

Light microscopy of thin-sections from resin embedded cell samples, showed cytopathic effects of the virus. At 24 hours post-infection, many cells lose their typical elongated shape of uninfected cells (*Fig. S2A*) and become roundish and rich in plasma membrane extroflections (*Fig. S2B*).. After 48 hours from the infection cell morphology further changed dramatically. Most cells appeared swollen and numerous cytoplasmatic vacuoles were visible inside them; in contrast, other cells appeared dark colored suggesting that cell shrinkage occurred (*Fig. S2C,D*).. Transmission electron microscopy analysis at 24 hours post-infection showed several round shaped cells, with prominent presence of filopodia at the plasma membrane (*Fig. 1B*).. Many mature viral particles were visible budding at the cell surface (*Fig. 1B,C*).. Inside the cells, SARS-CoV-2 particles were detected in virus containing compartments (VCC) that were with different size and shape (*Fig. 1E,F*).. Group of virions were enclosed in single membrane vacuoles, similar to endosomes (*Fig. 1E*).. Other vesicles, small in size, containing single viral particles, resembled the “spherules” described for other coronaviruses (*Fig. 1F*) [Refs 16,17]. At 48h post-infection most cells showed strong signs of degeneration and many were clearly dying (*Fig. 2*).. Some cells showed extensive vacuolization of the cytoplasm and depletion of all organelles (*Fig. 2A*).. Vacuoles containing viruses were still present in necrotic cells (*Fig. 2B*).. Some cells seemed to die with morphological features of both apoptosis and necrosis, in which condensed cellular contents was dispersed by means of plasma membrane leakage. Free released viruses were also observed associated with cell remnants (*Fig. 2C,D*)..

The most striking finding we observed in infected cells was the presence of numerous lipid droplets (LDs), with variable size and morphology (*Fig 2E,F*).. Some LDs showed homogeneous content, the typical feature of lipid storage without encompassing membrane (*Fig. 2E*).. Other droplets presented an external dark membrane (*Fig. 2E,F*) and were identified as lipolysosomes described in humans with abnormalities in lipid metabolism [17]. Mitochondria in contact with the lipid droplets were often found (*Fig. 2E*).. In most cells mitochondria appeared altered and display swollen cristae (*Fig. 2F*).. Of note, virus particles were also found associated with lipolysosomes suggesting that they can play an important step in virus assembly (*Fig. 2F*)..

EM findings - SARS-CoV-1 infected Vero cells.

During the first 24 hours post SARS-CoV-1 infection, Vero cells also showed modification of plasma membrane, which became enriched in filopodia and extroflections associated to the presence of numerous virus particles (*Fig. 3A*).. At 48h post-infection appearance of vacuoles and roundish of cells was displayed. Some infected cells showed the formation of large septa, resulting in a more dramatic compartmentalization of cytoplasm compared to SARS-CoV-2 (*Fig. 3B*).. Cytopathic effects in SARS-CoV-1 infected cells resulted in both apoptotic (*Fig. 3B*) and necrotic cell death (*Fig. 3C*).. Large vacuoles containing virus particles, resembling dilated spaces of endoplasmic reticulum were also detected (*Fig. 3D, S3A*).. Mitochondria displayed loss of their typical morphology, they appeared swollen with progressive cristae disappearance, resulting in the formation of vesicles, which occasionally still maintain mitochondrial matrix into the lumen. Virus particles were observed inside these vesicles, lining the

membrane or in the process of pinching off (*Fig. 3E*).. Virus particles were also found in deep association with particular multilamellar structures (*Fig. 3F*).. These structures were thought to derive from the rER, since ribosome-carrying membrane were observed in close continuity (*Fig. S3B*).. We didn't observe the presence of lipid droplets at any time of infection.

A comparative of the main features of the SARS-CoV-2 vs the SARS-Cov ultrastructural feature is shown in (*Fig 6*).

Histopathological examination of Lung tissue from COVID-19 patients.

In order to evaluate whether SARS-CoV-2 exerts similar cytopathic effects *in vivo*, we analyzed lung tissues obtained at autopsy from 20 COVID-19 patients. Histopathological analysis showed diffuse alveolar damage with hyaline membranes, fibrinous exudate, and inflammatory infiltrate (*Fig. 4A*).. Damage of alveolar epithelium was associated with the presence of reactive type II pneumocyte, characterized by hyperplasia, amphophilic cytoplasm, large nuclei and prominent nucleoli (*Fig. 4B*).. Type II pneumocytes showed increased detachment from the alveolar walls and displayed signs of degeneration consisting in highly vacuolated cytoplasm and nuclear changes, making the nucleus difficult to distinguish (*Fig. 4C,D*).. The immunohistochemistry anti-coronavirus revealed a focal distribution of the positivity, restricted to the activated type II pneumocytes (*Fig. 4E,F*).. It is important to note that absence of immunoreactivity to CD68 of these altered cells excluded that they were macrophages (*Fig. 4G,H*)..

Electron Microscopic examination of Lung tissue from COVID-19 patients.

Lung tissues showed the presence of SARS-CoV-2 virus inside type II pneumocytes (*Fig. 5A*).. As found in cultured infected cells, virions were observed enclosed in single layered cytoplasmic compartments of variable size, containing numerous viral particles, or as sole particles into the "Spherules" (*Fig. 5A,B*).. The pneumocytes showed altered morphological features, for example the nucleus appeared with finely and uniformly dispersed chromatin (*Fig. 5A*) or with convoluted profile and marginated chromatin alternated to cleared regions (*Fig. 5E*).. Those cells displayed organelles injury comparable with those observed in SARS-CoV-2 infected cells. The pneumocytes showed the presence of numerous vacuoles and swollen mitochondria (*Fig. 5A-D*).. The rough endoplasmic reticulum and free ribosomes, (which are typically abundant in type II pneumocytes, due to the production of surfactant), in the infected cells were respectively enlarged and compartmentalized (*Fig. 5C,D,F*).. Of note, in agreement to what observed in cultured cells, the infected type II pneumocytes showed unusual presence of abundant lipid droplets (LDs) (*Fig. 5E,F*)..

All SARS-CoV-2 infected cells ultimately died. Dying pneumocytes had morphological features which did not resemble neither necrosis or classical apoptosis. Some cells displayed partially condensation together with plasma membrane leakage and release of the cellular contents (*Fig. 5E*).

Figure 6 highlights that SARS-CoV-2 infection induces the same cytopathic effects both *in vitro* and *in vivo*.

Discussion

Three coronaviruses (CoVs) have crossed the species barrier to cause lethal zoonotic respiratory diseases in humans in the past 2 decades: Severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003, Middle-East respiratory syndrome coronavirus (MERS-CoV), in 2012 and the SARS-CoV-2 in 2019 [Refs 8, 18]. Coronaviruses are positive strand RNA viruses, that display a spherical morphology and spike glycoproteins projections on their surface which give them the typical crown-like shape under the electron microscope [Ref 19]. Since the mid-1960s, seven known human coronaviruses have been identified which involve the upper respiratory tract and the gastrointestinal tract, and generally cause mild diseases [Ref 20]. As other viruses, CoVs display an envelope that is formed by a lipid bilayer derived from the host cell membranes and for this reason intracellular membrane play a key role for coronaviruses replications. Coronavirus replication complexes, similar to other RNA viruses, appear to be anchored to membrane structures known as “viral factories”, derived from extensive modification of cell compartments [Ref 21]. These membranous structures not only harbor viral proteins but also contain a specific array of hijacked host factors, which collectively orchestrate a unique lipid micro-environment optimal for coronavirus replication [Ref 21–24]. Ultrastructural studies on Coronavirus genera have revealed that alpha- and beta-coronaviruses formed clusters of the double-membrane vesicle (DMV), sometimes linked by a convoluted membrane [Ref 25], whereas the gamma-coronavirus IBV induced extensive paired membranes and smaller 60–80 nm spherules in addition to the DMVs [Refs 25–27]. In a recent study, it has been demonstrated that during human coronavirus infection the cell lipid profile is significantly altered [Ref 28].

An observation in our study regarding SARS-CoV-2 is that there is the striking difference compared to SARS-CoV-1, consisting in the presence of numerous lipid droplets. In SARS-CoV and MERS-CoV, host lipids have been reported to be linked most to pathogenesis. In particular, the lipid rafts are required for cellular entry [Refs 29, 30]. Some (+)RNA viruses exploit lipid droplets (LDs) to acquire lipids for membrane or energy production to support their replication [Ref 31]. Lipid droplets found in SARS-Cov-2 infection, both *in vitro* and in type II pneumocytes, appear similar to those known to occur in hepatocytes as a consequence of HCV infection. Lipid droplets with typical features of lipid storage, without encompassing membrane and translucent omogeneous appearance, were often observed. Other vesicles with the characteristics similar to lipolysosomes were also present, with external membrane and whorls. In Vero cells viral particles were also found associated with lipolysosomes suggesting that they can play a role in virus assembly. Another important observation concerns mitochondria. A number of mitochondria were in close contact with lipids droplets. These contacts site have recognized as a key feature of lipid dynamics [Ref 32]. The proximity of mitochondria and lipid droplets is necessary for the

ATP production, via β -oxidation. Recent findings described host lipid metabolic remodelling associated with human-pathogenic propagation of HCoV-229E, suggesting that lipid metabolism regulation could be a common event for coronavirus infections [Ref 28].

Modulation of host lipid metabolism has been reported to be necessary for replication of virus, such as hepatitis C virus (HCV), and picornaviruses [Refs 33,34]. Several studies demonstrated that targeting host lipid metabolism by statins, allow to suppress viral replication of many positive-strand RNA viruses, such as Hepatitis C virus, Dengueviruses, Japanese encephalitis virus, West Nile virus and influenza A virus. Statins, are able to destabilize lipid rafts involved in the viral replication phases, as they constitute packets of vesicles capable of concentrating virus replication factors [Refs 35,36].

We showed that type II alveolar epithelial cells appear to be the main target of the SARS-CoV-2. Whilst SARS-CoV-2, induces rapid dramatic ultrastructural changes and death in the host cells, the morphological features do not correspond to the activation of apoptosis, which is, by contrast, the main mechanism of cell death induced by SARS-CoV infection [Refs 37,38]. Our results on SARS-CoV-2 infected cells suggested that a distinct type of cell death, with morphological features of both apoptosis and necrosis, namely Pyroptosis, could be induced by the virus [Ref 39]. Our hypothesis for an involvement of pyroptosis in pathogenesis of COVID-19 is in line with increased IL-1 β in the serum of patients infected with SARS-CoV-2 recently described [Ref 40]. COVID-19 is associated with a respiratory illness that may lead to severe pneumonia and acute respiratory distress syndrome (ARDS). Of note, in the pathogenesis of ARDS pyroptosis may play an important role [Ref 41].

In conclusion, our findings highlight several ultrastructural cell changes induced by SARS-CoV-2 infection. Of note, similar changes were found in cultured infected Vero cells and in lung type II pneumocytes from patients, demonstrating that they represent the real profile of cytopathogenic events induced by SARS-CoV-2. Since the alveolar type II pneumocytes are multifunctional cells which play a fundamental role in barrier function, in alveolar fluid balance, coagulation/fibrinolysis, and host defence [Refs 42, 43] the results showed here could open interesting perspectives for future therapeutic approaches. In particular, the SARS-CoV-2 induced generation of lipid droplets suggests that clinical studies, to assess the efficacy of statin on COVID-19 patient, could open yet unconsidered therapeutic perspective.

Declarations

Acknowledgments

This work was supported in part by Horizon 2020 grants for EU project 101003544-CoNVat and EU project 101003551 - EXSCALATE4CoV. Grants from the Ricerca Corrente e Finalizzata from the Ministry of Health, AIRC (IG2018-21880 to M. P.), Regione Lazio (Gruppi di ricerca, E56C18000460002, to M. P.). M. P. also acknowledges the support of the grant from the Russian Government Program for the Recruitment of the Leading Scientists into the Russian Institutions of Higher Education (14.W03.31.0029

to M. P.). Professors Ippolito and Sir Zumla are co-PIs of the Pan-African Network on Emerging and Re-Emerging Infections (PANDORA-ID-NET – <https://www.pandora-id.net/>) funded by the European and Developing Countries Clinical Trials Partnership the EU Horizon 2020 Framework Programme for Research and Innovation. Sir Zumla is in receipt of a National Institutes of Health Research senior investigator award. Roberta Nardacci is Professor at Saint Camillus International University of Health and Medical Sciences in Rome.

Author contributions

All authors ideated the autopsy and EM studies and contributed to data interpretation and writing of the manuscript. R. N., M. P. and L. F. designed the project and performed the ultrastructural analysis. F. C., C. C., D. L., G. M. and S. M. isolate the 2019-nCoV/Italy-INMI1 virus and performed cell infection experiments. F. D. N. and D.C. collected autoptic specimens and performed histopathological analysis. M. R. C., A. Z., G. I. and M. P. take responsibility for the integrity and the accuracy of the data analysis. R. N., M. P. and L. F. discussed the results and wrote the paper. All authors contributed approved the final version.

Author declarations:

The authors declare no competing interests.

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Figures

Figure 1

Electron microscopy images of SARS-CoV-2 virus and infected Vero cells. (A) Negative staining electron microscopy micrographs of SARS-CoV-2 particle. The virion display a spherical shape, on the surface cone-like shaped spikes are visible (white arrow). (B-D) Infected cells shows numerous viruses budding from the plasma membrane (arrowheads) especially found along microvillous projections (arrows). Many lipid droplets (LD) and lipolysosomes (LL) were visible (arrow) (D). (E) A great number of vacuoles are present in the cell cytoplasm, many of which contained viral particles (arrows). (F) Other vesicles, small in size, contain single viral particles, resembled the “spherules” described for other coronaviruses (arrows). Viruses are budding from the plasma membrane (arrowheads). Numerous free ribosomes are diffused in the cell cytosol. N, nucleus; m, mitochondria; LD, lipid droplets; LL, lipolysosomes. Scale bars: A= 100 nm; B,D,F =1um; C,E =200 nm.

Figure 2

Electron microscopy features of SARS-CoV-2-infected dying cells. (A,B) A dying cell shows an advanced stage of degeneration. Numerous viruses bud from the cell membrane (arrowheads). The cytoplasm results empty due to the presence of a high number vacuoles, some of which containing viral particles (arrows). The nucleus shows condensed areas. (C,D) Cell remnants showing viral particles outside (arrows). (E) Lipid droplets (LD) inside infected cells, some of which are in contact with mitochondria

(arrows). Smallest lipolysosomes (LL) with external membrane and whorls are detected. Mitochondria (m) show swollen cristae. (F) Image showing lipolysosomes (LL) containing viral particles (arrowhead). The white space, visible in the figure, is due to the not well preserved lipolysosome ultrastructure. N, nucleus; m, mitochondria; LD, lipid droplets; LL, lipolysosomes;. Scale bars: A,E,F = 1um; B,E,F= 200 nm.

Figure 3

Electron microscopy micrographs of SARS-CoV-1 infected Vero cells. (A) Infected cells show a rough aspect, and many microvillous projections are visible all over the cell surface (arrows). Numerous virus particles are visible budding at the cell membrane or in the extracellular space (arrowheads). (B) Cytopathic effect of viral infection generates condensed cytoplasm and a number of them undergo apoptotic cell death (arrow); necrotic (C) cellular lysis with release of cell material with associated virus particles is also observed. (D) Cytoplasmic vesicles with accumulated viral particles are present near the plasma membrane of infected cells (arrows). (E) Morphological modifications of mitochondria (m) are shown. The organelles display swelling with a reduction in membrane cristae amount, leading to the formation of vesicles (arrows). Virus particles are visible inside these vesicles (arrowheads). (F) SARS-CoV-1 causes the formation of multilamellar structure: at the periphery of the structure ribosome-carrying membrane are shown (arrowheads). N, nucleus; m, mitochondria. Scale bars: A,B,C = 1 μ m; D-F = 200 = nm.

Figure 4

Histopathological changes of lung tissue from COVID-19 patients. (A,B) EE staining from lung tissue shows diffuse alveolar damage, with intra-alveolar inflammation, fibrin, hyaline membranes. (B) Hyperplasia of type II pneumocyte, characterized by amphophilic cytoplasm, large nuclei and prominent nucleoli is visible (arrows). (C,D) Type II pneumocyte showing signs of degeneration characterized by large nuclei, with fine and uniformly dispersed chromatin and cytoplasm vacuolization (arrows). (E,F)

Immunohistochemistry anti-coronavirus revealed a focal distribution of the positivity in type II pneumocytes (arrows). (G,H) Immunolabeling of CD68-positive macrophages (arrowheads) allows the clear identification of type II pneumocytes (arrows). Scale bars: A = 14 μ m; B-H = 7 μ m

Figure 5

SARS-Cov-2 detecting on lung tissue from COVID-19 patients by transmission electron microscopy. (A,B) SARS-CoV-2 particles are detected in type II pneumocytes. Lamellar bodies, containing surfactant are

visible (LB). Viruses are localized in virus containing compartments (arrows). Other vesicles, very small in size, contain single viral particles (arrowheads). (C) Mitochondria (m) display swelling with a reduction in membrane cristae amount. (D) Rough endoplasmic reticulum, appear very enlarged (rER). (D) Numerous vacuoles are present in the cell cytoplasm (arrows). (E,F) Abundant lipid droplets (LD) are present inside infected cells. Free ribosomes are present in the cell cytosol, many of which are compartmentalized (arrow). N, nucleus; m, mitochondria; rER, rough endoplasmic reticulum, LD, lipid droplets; LB, lamellar bodies. Scale bars: A,E=1um; B,C,D,F=200 nm.

		Cultured cells SARS-CoV	Cultured cells SARS-CoV-2	Type II pneumocytes from COVID-19 patients
Filopodia	Numerous protrusions of the plasma membrane	Numerous protrusions of the plasma membrane	Numerous protrusions of the plasma membrane
Virus Containing Compartments (VCC)	VCC containing numerous viral particles	VCC containing numerous viral particles and small vesicles containing single particles	VCC containing numerous viral particles and small vesicles containing single particles
Lipid Droplets	Absent	Presence of numerous lipid droplets and lipolysosomes	Presence of numerous lipid droplets
Mitochondria	Mitochondria display swelling with a reduction in membrane cristae amount, leading to the formation of vesicles	Mitochondria shows dilated cristae	Mitochondria display swelling with a reduction in membrane cristae amount
Multilamellar structures	Multilamellar structure derived from endoplasmic reticulum	Absent	Absent
Cell Death	Cell death mainly dependent on the activation of apoptosis	Dying cells show morphological features of both apoptosis and necrosis	Dying cells show morphological features of both apoptosis and necrosis

Figure 6

Main differences in the ultrastructural cytopathogenesis induced by SARS-Cov-2 and SARS-Cov infection in cultured Vero cells, and analogue between in vitro and in vivo.

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