**INTRODUCTION**

**Coronavirus disease 2019** (**COVID-19**) is an infectious disease caused by the [severe acute respiratory syndrome coronavirus 2](https://en.wikipedia.org/wiki/Severe_acute_respiratory_syndrome_coronavirus_2) (SARS-CoV-2). The disease has spread globally since 2019, resulting in the [2019–20 coronavirus pandemic](https://en.wikipedia.org/wiki/2019%E2%80%9320_coronavirus_pandemic). The [deaths per number of diagnosed cases](https://en.wikipedia.org/wiki/Case_fatality_rate) is estimated at between 1% and 5% but varies by age and other health conditions. Coronaviruses are single stranded, positive sense RNA viruses, which induce the rearrangement of cellular membranes upon infection of a host cell. This provides the virus with a platform for the assembly of viral replication complexes, improving efficiency of RNA synthesis. The membranes observed in coronavirus infected cells include double membrane vesicles.

The infection is spread from one person to others via [respiratory droplets](https://en.wikipedia.org/wiki/Respiratory_droplets), often produced during coughing and sneezing. Time from exposure to onset of symptoms is generally between 2 and 14 days, with an average of 5 days. The standard method of diagnosis is by [reverse transcription polymerase chain reaction](https://en.wikipedia.org/wiki/Reverse_transcription_polymerase_chain_reaction) (rRT-PCR) from a [nasopharyngeal swab](https://en.wikipedia.org/wiki/Nasopharyngeal_swab) or throat swab. The infection can also be diagnosed from a combination of symptoms, risk factors and a chest [CT scan](https://en.wikipedia.org/wiki/CT_scan) showing features of pneumonia.

The virus causes respiratory illness (like the flu) with symptoms such as a cough, fever and in more severe cases, pneumonia. You can protect yourself by washing your hands frequently and avoiding touching your face.

**SYMPTOMS**

Common symptoms include [fever](https://en.wikipedia.org/wiki/Fever), cough and shortness of breath. Muscle pain, [sputum](https://en.wikipedia.org/wiki/Sputum) production and sore throat are less common symptoms. While the majority of cases result in mild symptoms, some progress to [pneumonia](https://en.wikipedia.org/wiki/Pneumonia) and [multi-organ failure](https://en.wikipedia.org/wiki/Multi-organ_failure).

More rarely, the disease can be fatal. Older people, and people with other medical conditions (such as asthma, diabetes, or heart disease), may be more vulnerable to becoming severely ill.

**COVID-19 Pathophysiology**

COVID-19 is caused by SARS-CoV-2, a beta corona virus. It is comprised of a single-stranded ribonucleic acid (RNA) structure that belongs to the Corona virinae subfamily, part of the Corona viridae family. Sequence analysis of SARS-CoV-2 has shown a structure typical to that of other coronaviruses and its genome has been likened to a previously identified coronavirus strain that caused the SARS outbreak in 2003. Structurally, the SARS coronavirus (SARS-CoV) has a well-defined composition comprising 14 binding residues that directly interact with human angiotensin- converting enzyme2. Of these amino acids, 8 have been conserved in SARS-CoV-2. In humans, corona viruses were thought to cause mild respiratory infections until the identification of SARS-CoV and MERS coronavirus (MERS-CoV).

Autophagy

Autophagy is a cellular pathway for self-degradation. The pathway allows a cell to degrade long-lived proteins, aggregated proteins and organelles during periods of starvation to provide nutrients for continued cellular processes, as well as playing an important role in cellular homeostasis, ageing and development. In addition, dysregulation of autophagy plays an important role in the development of some cancers. During autophagy, regions of the cytoplasm become engulfed into double membrane bound vesicles termed autophagosomes. These vesicles then fuse with late endosomes/lysosomes, where the contents are degraded by lysosomal proteases.

Coronavirus Replication and Autophagy

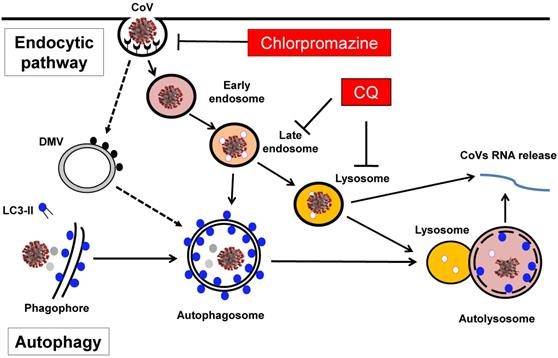
The presence of DMVs in coronavirus infected cells suggested that this group of viruses might, like other positive sense RNA viruses, utilise the autophagy pathway to generate the membrane structures required for replication. Initial work showed that MHV infection induced autophagy [50]. Nsp8 co-localised with LC3 throughout infection and the nucleocapsid protein N co-localised with LC3 early in infection, but this decreased over time. In addition, MHV replication was markedly reduced in ATG5−/− embryonic stem cell lines but virus titre was rescued in the presence of an ATG5 expressing plasmid [50]. Further work using SARS-CoV again showed co-localisation between nsp8 and LC3 [51]. However, work by others using bone marrow derived macrophages lacking ATG5 or ATG5−/− primary murine embryonic fibroblasts (MEFs), showed that MHV replication does not require either an intact autophagy pathway or conversion of LC3I to LC3II. They did however confirm that SARS-CoV nsps co-localised with LC3 [52]. Recently, Schneider et al. demonstrated that SARS-CoV replication could also occur in ATG5−/− MEFs, indicating no requirement for a complete autophagy pathway [53]. Interestingly, it was observed that virus replication was unaffected by the induction of autophagy in wild type MEFs [53]. Finally, de Haan et al. were unable to show co-localisation between MHV nsp8 and GFP-LC3 [54], and Snijder et al. were unable to show co-localisation between SARS-CoV nsp3 and either endogenous LC3 or GFP-LC3A, GFP-LC3B or GFP-LC3C [9]. Despite the lack of clarity with regard to the requirement for autophagy during coronavirus infection, all studies did indicate that LC3 became punctate upon coronavirus infection, suggesting an induction of autophagy [9,50–52,54]. Consistent with previous observations, recent experiments performed in mammalian cells using IBV showed that this avian coronavirus is also capable of inducing autophagy during infection [55]. Here, further work was performed and individual expression of viral nsp6 was also capable of inducing autophagy, whereas nsp4 and nsp10 were not. Nsp6 induced autophagosomes fused with LAMP1 labelled lysosomes and autophagosomes were susceptible to wortmannin treatment. This indicates that bone fide autophagy was induced. Interestingly, nsp6 homologues from SARS-CoV, MHV and arterivirus porcine reproductive and respiratory syndrome virus (PRRSV), also induced autophagy.

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The mechanism for the induction of autophagy was not determined, although it was shown not to be by induction of ER stress, MTOR inhibition or via sirtuin 1. In addition, it was not confirmed whether nsp6 was responsible for the induction of autophagy in virus infected cells. However, in agreement with previous work, virus infection was not inhibited by the knockdown of ATG5 expression [55]. In other work, it has been suggested that whilst endogenous LC3 could co-localise with MHV RTCs, GFP-LC3 showed significantly reduced co-localisation [56]. This may provide an explanation for some of the earlier experimental discrepancies. Further work in this study demonstrated that LC3 puncta were still observed in MHV infected ATG7−/− cells, where LC3I to LC3II conversion cannot occur and also in cells expressing LC3 that cannot be processed to LC3II (LC3 G120A). In addition, although the absence of the complete autophagy pathway due to a lack of ATG7 did not alter MHV replication, reduced expression of LC3 by RNAi did significantly reduce virus replication. Furthermore, viral protein expression could be rescued in the presence of LC3 G120A [56]. This demonstrated that MHV replication does not require the complete autophagy pathway, or conversion of LC3I to LC3II, in agreement with previous work, but instead required LC3I [52]. Interestingly, MHV RTCs co-localise with markers for cellular EDEMosomes [56,57], vesicles involved in ER associated protein degradation (ERAD) tuning and knockdown of EDEMosome cargo receptor SEL1L reduced virus replication [57,58]. In the ERAD pathway, unfolded proteins are removed from the ER and targeted for degradation. However, under normal conditions, the ERAD machinery must be regulated to prevent premature removal of proteins before they have been folded [59]. During ERAD tuning, parts of the ERAD machinery are removed from the ER in EDEMosomes to down regulate the pathway [57,58,60]. LC3I is recruited to EDEMosomes by transmembrane protein SEL1L [57] and possibly acts as a coat protein [60]. This work has highlighted a role for LC3 as part of the ERAD tuning pathway, but not autophagy, in the replication of MHV. Whether this pathway is involved in the replication cycles of other coronaviruses remains to be determined.

**Involvement of the endocytic pathway and autophagy in the entry and replication of CoVs in host cells.** Entry of CoVs into the host cells is mainly mediated by the endocytic pathway, meanwhile the autophagy has also been implicated in the viral replication in the cells, a process partly related to the formation of DMV in the host cells. As a result, several groups of inhibitors including the lysosomotropic agents such as CQ and inhibitors for clathrin-mediated endocytosis such as chlorpromazine have been proposed to have therapeutic efficacy against CoVs-induced diseases including COVID-19.



**Implication of autophagy in CoVs infection**

In the past one and a half decades, the implication of autophagy in CoV infection has attracted substantial attention, probably due to the SARS outbreak in 2002-2003 and the emerging field of autophagy research at the same period. At present, various reports have converged onto two important questions: whether CoV induces autophagy and whether the autophagy machinery or ATG proteins are involved in the infection and replication of CoVs. The first report demonstrating the involvement of autophagy in viral replication was based on MHV [[24](https://www.ijbs.com/v16p1724.htm#B24)], in which the authors made several important observations. First, MHV induced the formation of double-membrane vesicles (DMVs), with resemblance to autophagosome, a hallmark of autophagy. Second, the viral replication complexes at DMVs co-localized with the autophagy proteins, LC3 and ATG12. Third and more importantly, MHV was impaired in *ATG5* knockout embryonic stem cells. Therefore, the authors concluded that autophagy is implicated in the formation of DMV as well as in the replication of MHV [[24](https://www.ijbs.com/v16p1724.htm#B24)]. In a follow-up study, the same group also examined the SARS-CoVs and found similar colocalization of the key viral replication proteins with endogenous LC3, a protein marker for autophagosome [[25](https://www.ijbs.com/v16p1724.htm#B25)], suggesting a similar function of autophagy in the replication of SARS-CoVs. Cottam *et al* used another CoV (infectious bronchitis virus, IBV) and found that one of the key viral replicase protein nsp6 is capable of inducing autophagy [[26](https://www.ijbs.com/v16p1724.htm#B26)]. Notably, this nsp6 also presents in MHV and SARS-CoV, and thus it would be of interest to further test the effects of nsp6 in these two CoVs on autophagy.

However, several subsequent studies have challenged the notion that autophagy is implicated in CoV infection. For instance, in Vero cells infected with SARS-CoVs, Snijder *et al* failed to detect colocalization of LC3 or GFP-LC3 with the viral replication- transcription complexes of SARS-CoV examined using immunofluorescence staining [[27](https://www.ijbs.com/v16p1724.htm#B27)]. Further studies also demonstrated that either ATG5 or ATG7, two of the key autophagy proteins in control of autophagosome biogenesis, is not required for viral replication in cells infected by MHV [[28](https://www.ijbs.com/v16p1724.htm#B28), [29](https://www.ijbs.com/v16p1724.htm#B29)] or by SARS-CoVs [[30](https://www.ijbs.com/v16p1724.htm#B30)]. In those studies, cells with deletion of either ATG5 or ATG7 failed to impair the viral replication rate. Similarly, virus infection was not inhibited by the knockdown of ATG5 [[26](https://www.ijbs.com/v16p1724.htm#B26)]. Thus, all these observations suggest that the autophagy machinery is not directly implicated in the viral replication process.

Intriguingly, there is evidence suggesting the possible inhibitory effect of CoV on the autophagy process. For instance, a study using SARS-CoV and MERS-CoV in HEK293T, HeLa and MCF-7 cells found that overexpression of membrane-associated papain- like protease PLP2 (PLP2-TM) of SARS-CoV and MERS-CoV led to blockage of autophagosomes- lysosomes fusion and suppression of the autophagic flux [[31](https://www.ijbs.com/v16p1724.htm#B31)]. Consistently, a more recent report found that MERS-CoV blocks the fusion of autophagosomes and lysosomes and induction of autophagy reduces the replication of MERS-CoV [[32](https://www.ijbs.com/v16p1724.htm#B32)]. Thus, it appears that there is certain type of interplay between the autophagy machinery and CoVs, and the exact nature of such interaction remains to be further elucidated.

Taken together, it is still controversial whether and how autophagy is implicated in the infection of CoVs. The discrepancies in the literature is probably due to the different viruses used, different cells tested and even the different techniques used in study of autophagy.

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