Undergraduate Journal of Experimental Microbiology and Immunology (UJEMI) Vol. 7:1-8

UJEMI PERSPECTIVES

Syncytia Formation: A trademark of severe SARS-CoV-2 infection and a potential target for novel drug therapies

Loujain Bilal

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY Coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, is characterized by its symptoms and complications primarily affecting the upper respiratory tract. Infected multinucleated syncytial pneumocytes have been observed in severe COVID-19 cases, alongside lung damage and lymphocytopenia. Syncytia formations allow the virus to evade the immune system, disseminate without cell exocytosis and cause cytopathicity and cell death. The mechanism of cell-cell fusion in SARS-CoV-2 infected cells is well documented as it relies on the same machinery as cell entry. The free spike (S) proteins localized at the cell membrane interact with ACE2 receptors and host proteases on neighbouring cells causing the cell membranes to fuse. However, the biological composition of these multinucleated cells is not well characterized. Furthermore, how these multinucleated syncytia are interacting with their surrounding environment to cause the physical manifestations that are observed in COVID-19 patients is not yet fully understood. At present, there are no antivirals drug that have been developed or in use that directly target syncytia formation in SARS-CoV-2 and other syncytia-inducing viruses. This makes syncytia inhibition an overlooked yet promising area for new drug therapy development. Naturally, these shortcomings lead to the following questions that this article will focus upon 1) what the cellular and molecular composition of multinucleated syncytia is and how do they function biologically, and 2) how syncytia can be targeted to produce broad spectrum multidrug antivirals. A better understanding of the cellular composition and biological functions of syncytia can allow for a novel syncytia targeted antiviral that can prevent or reduce severe lung damage and other syncytial manifestations. Cell-cell fusion is not unique to SARS-CoV-2 as many other enveloped viruses such as respiratory syncytial virus (RSV) and Middle East respiratory syndrome related coronavirus (MERS-CoV) are known to induce syncytia in infected cells. Therefore, the impact of this research extends beyond SARS-CoV-2 and can pave the way for broad spectrum antivirals that target syncytia formation in other enveloped viruses. This could potentially reduce the burden that syncytia forming viruses have on public health, including the ongoing COVID-19 pandemic.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the COVID-19 pandemic. The first case of COVID-19 emerged in late 2019 and soon after, the world was launched into a pandemic and a rapidly developing public health emergency. As of 2023, COVID-19 has caused a staggering 6.8 million deaths and infected more than 800 million individuals worldwide (1). SARS-CoV-2 belongs to the beta coronavirus family, a clade of viruses that have caused previous outbreaks including the SARS-CoV outbreak in 2001 and Middle East respiratory syndrome-related coronavirus (MERS-CoV) outbreak in 2012 (2). Collectively, and particularly in recent years, these viruses have placed a heavy burden on public health (2). Like other coronaviruses, SARS-CoV-2 enters and infects the upper respiratory tract of its host, using the viral spike glycoprotein (S) to facilitate viral entry. As a result, SARS-CoV-2 is well characterized by its respiratory related symptoms including alveolar edema, lung thrombosis and in severe cases, chronic respiratory diseases (CRDs) and acute respiratory distress syndrome (ARDS) (3, 4). Although viral dissemination allows SARS-CoV-2 to infect other regions of the body including the central nervous system (CNS) and gastrointestinal tract, the focus of this article

Published Online: September 2023

Citation: Bilal. 2023. Syncytia Formation: A trademark of severe SARS-CoV-2 infection and a potential target for novel drug therapies. UJEMI Perspectives 7:1-8

Editor: François Jean (Ph.D.), University of British Columbia

Address correspondence to: https://jemi.microbiology.ubc.ca/

will be on the upper respiratory tract and its peculiar and adverse formation of multinucleated pneumocytes, known as syncytia (5).

Syncytia is defined as cellular structures containing two or more nuclei formed by the fusion of uninuclear cells (6). Syncytia formation is commonly seen in infections caused by bacterial pathogens, parasites and viruses (7). However, syncytia are also naturally occurring in many organisms including mammals and are involved in processes such as fertilization, immune responses, skeletal muscle cell development and placenta formation (6, 8). Various enveloped viral families are known for their syncytial capability including Herpesviridae, Paramyxoviridae, Coronaviridae and Retroviridae (7). Syncytia express high levels of viral antigens and allow for viral dissemination, evasion of the immune system, cytopathicity and cell death (2, 7). Consequently, syncytia formation in virus infected cells induce major conformational changes and inflammatory responses that can have drastic and negative physiological impacts on its host (2). In 2021, significant findings were made when lung tissue biopsies taken from SARS-CoV-2 infected patients hospitalized for CRDs were histologically analyzed. Researchers observed that infected pneumocytes had an abnormal morphology and that in 90% of the patient biopsies, there was frequent multinucleation (9). These observed syncytia formation are a direct result of the fusogenic capabilities of the S protein, a viral fusogen, as the expression of the S protein alone is sufficient to produce syncytium (10). This occurs when newly translated and folded free spike proteins are not packed into virions through the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and are instead trafficked to the plasma membrane through the trans-Golgi network in secretory vesicles. At the plasma membrane, the furin-cleaved spike protein can bind to an angiotensin-converting enzyme 2 (ACE2) receptor on a neighbouring cell and induce cell-to-cell fusion (11). Further research has identified that a four polybasic amino acid (P-R-R-A) insertion right before the S1/S2 cleavage site is responsible for the fusogenic capabilities of the SARS-CoV-2 spike protein (10). Furthermore, the bi-arginine motifs (R682 and R685) preceding the S1/S2 cleavage site were identified as integral to membrane fusion. Zhang et al. demonstrated that upon mutation of R683 or P681, wild type expression of syncytia formation was seen. Contrarily, upon mutation of R682 and R685, syncytia formation was significantly reduced (10). These findings explain the decreased pathogenicity of omicron compared to delta and the ancestral SARS-CoV-2 strains. The omicron variant has a histidine mutation at P681 and lysine mutation at N679, although individually these mutations increase fusogencity and transmissibility, the overall pathogenesis is decreased (12, 13). Beyond the fusion mechanism and its association to pathogenicity in human lungs, syncytia in SARS-CoV-2 infected cells and in general, are not well characterized or described.

PROPOSED RESEARCH QUESTIONS

Lung cell syncytia formation in severe COVID-19 patients compromises the functionality of the lung. It is associated with severe lung damage including thrombosis, alveolar damage, and fibrosis (9). However, very little is known about how syncytia induce the observed lung pathogenicity. Specifically, syncytial morphology and biological composition are not well characterized. Our understanding of how syncytia function and are internally organized is quite preliminary. Similarly, the changes in gene expression, uptake and secretion are not well documented in viral-induced syncytia. Therefore, understanding syncytia mechanisms of regulation and its phenotype is integral to building a better understanding of how SARS-CoV-2 can cause these symptoms and its overall pathogenicity. This gap in knowledge leads to the first research focus 1) What is the cellular and molecular composition of multinucleated syncytia and how they function biologically?

Additionally, previous research has established the importance of fusogencity to not only syncytia formation, but also in viral entry. The spike protein, a viral fusogen, allows SARS-CoV-2 to easily enter and infect new healthy cells expressing the ACE2 receptor. Syncytia formation has a clear and important role in the dissemination of SARS-CoV-2 and evasion of the host immune responses. These qualities make it an appealing target for antivirals. Furthermore, since cell-to-cell fusion involves similar host and viral components to cell entry, developing a combination antiviral therapy that targets syncytia formation could be advantageous to blocking multiple viral replication stages of SARS-CoV-2. Additionally, syncytia formation is not unique to SARS-CoV-2 as many other enveloped viruses can also

induce the formation of multinucleated cells (syncytia). Ergo, targeting this process has potential for the development of a broad-spectrum acting antiviral. Therefore, the second research focus of this manuscript is 2) How can syncytia be targeted to produce broad spectrum multidrug antivirals?

PROPOSED PROJECT NARRATIVE

What is the cellular and molecular composition of multinucleated syncytia and how do they function biologically? The morphology and organelle organization of syncytia is quite diverse and varies depending on the innate functions of the cell and the organism. However, very little research has focused on the morphology of SARS-CoV-2 induced syncytia. Therefore, the use of similar multinucleated cell models in other species is integral to establishing a preliminary understanding and hypothesis for what these syncytia may look like. Evidently, numerous nuclei are observed in syncytia ranging from 2 to 20, however many other studies recorded upwards of 50 nuclei (9, 14). Aside from quantity, the nuclei in syncytia were observed to have conformational changes in soybean root cells (14). A few studies looking at syncytia formation in multinucleated giant cells also observed a lock and key organizational pattern of the nuclei (15). Furthermore, the invagination of the nucleus membrane was observed in syncytia formed by chick embryonic fibroblasts (16). Finally, MGCs formed by fusion of macrophages showed an increase ability to phagocytose (17). Whether this increased phagocytotic ability extends to cells without an innate function to phagocytose is still unknown. However, the association of lymphopenia and increased cell in cell structures with syncytia formation in SARS-CoV-2 infected patients may indicate higher levels of phagocytosis (18). However, which cellular contents are kept in the syncytia from the merged cell and which structures undergo autophagy for energy remains unknown.

Similarly, the transcript and proteome of viral induced syncytia are not well identified or researched. However, preliminary knowledge can be gathered on what genes are expressed in syncytia by observing the change in gene regulation in syncytia caused by other parasites. For instance, a transcriptome analysis of syncytia induced by the cyst nematode, *Heterodera schachtii*, in Arabidopsis roots had compelling findings regarding gene regulation in syncytia. Firstly, the transcriptome of the syncytium was declared to be clearly different and unique from the surrounding root cells and all other organs of the plant (19). This suggests that syncytia, although are formed from the fusion of multiple cells, do not behave, and regulate genes like their constituents. Furthermore, genes related to high metabolic activity were upregulated including genes associated with translation and biosynthesis (19). Similarly, an increased number of ribosomes has been observed in SARS-CoV-2 infected cells exhibiting syncytia (20). The overall increase in metabolic activity coincides with the increased number of mitochondria seen in MGCs and mitochondrial swelling in SASR-CoV-2 infected cells (17, 20). Conversely, defense gene expression was downregulated (19). This may suggest that syncytia formation can reduce the hosts' ability to mount an adequate defense response against pathogens, although this would first need to be validated in viral-induced syncytia in animal cells.

Lastly, our knowledge of the syncytia secretome is surprisingly limited. Syncytia have been described as sinks of nutrients and understanding the secretion system in syncytia would provide a lot of insight into their functions and interactions with surrounding tissue as well as they self-sufficiency (19). Uncovering the molecules that are secreted and taken up by syncytia should be among the research areas focused on moving forward.

How can syncytia be targeted to produce broad spectrum multidrug antivirals? Syncytia formation was observed only in severe stages of COVID-19 and associated with lung damage, suspected to induce lymphopenia and a higher mortality rate (6). Consequently, developing an antiviral therapy directed at reducing syncytia formation in severe COVID-19 patients should be both a research and public health priority.

Furin Inhibitors. Furin is an integral host protease employed by SARS-CoV-2 to cleave its spike protein and induce syncytia formation. The SARS-CoV-2 spike protein is composed of two subunits, S1 and S2. N-terminus of the S1 subunit is responsible for binding with host cell receptor ACE2 (21). S2 is cleaved at two motifs by host proteases to facilitate viral entry. Furin-mediated endoproteolytic cleavage at the S1/S2 cleavage site induces conformational

rearrangements that reveal the S2' site for TMPRSS2-mediated cleavage. Subsequently, the cleavage at the S2' cleavage site reveals the fusion peptide and allows the release of viral RNA genome into the host cell. Furin can be found in the trans-Golgi network, on the plasma membrane or secreted extracellularly in cells (22). Consequently, the spike protein can be cleaved by furin as they are trafficked through the secretory pathway to the cell membrane from the trans-Golgi network. Once at the plasma membrane, the cleaved spike proteins can bind to ACE2 on a neighboring cell and induce cell membrane fusion. During fusion, cellular contents of both cells merge including the viral proteins and genome of the infected cell thus, increasing viral dissemination and infection in the host. Evidently, furin mediated cleavage of the spike protein is integral to syncytia formation making it an appealing and promising target for antivirals (21). Although there is yet to be an FDA approved antiviral therapy that targets furin, research has shown promising evidence that the inhibition of furin reduces S-mediated syncytia formation and viral pathogenesis (21). The general mechanism of furin-mediated syncytia inhibition is outlined in Figure 1. Among the studied furin inhibitors is a

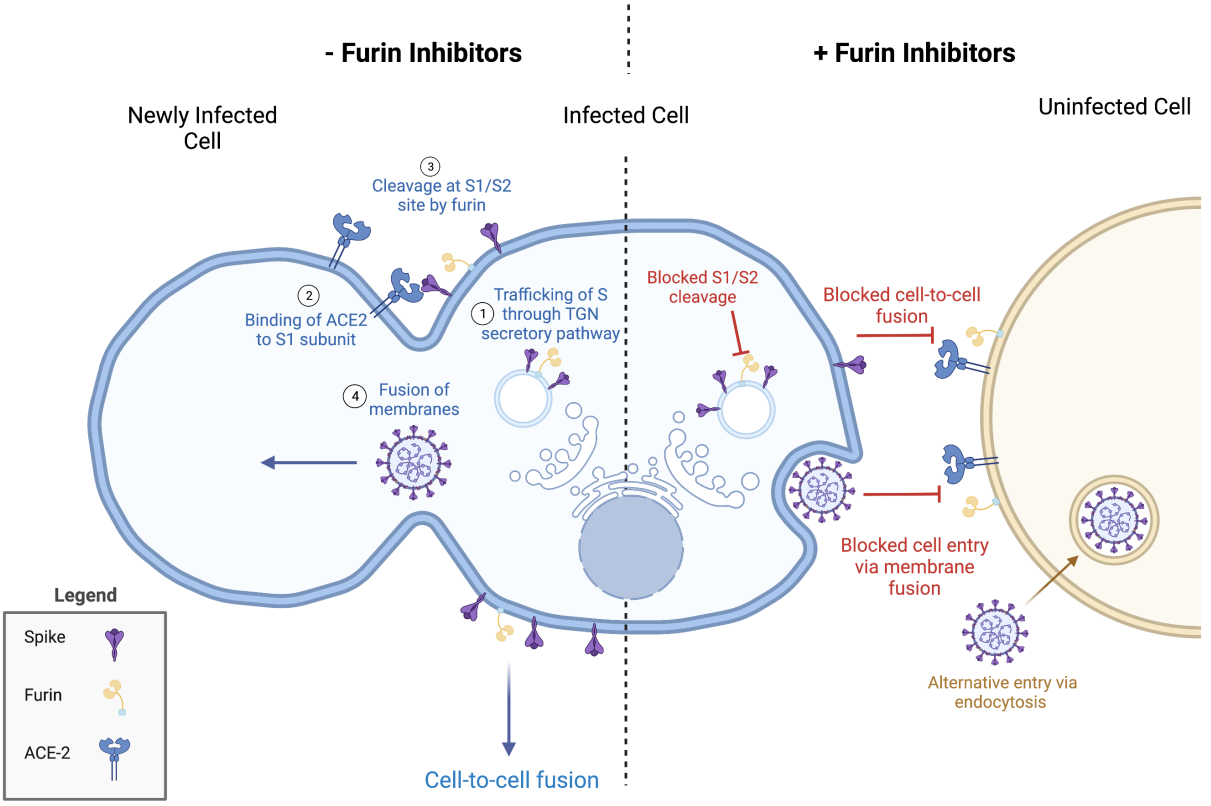

FIG. 1 Overview of SARS-CoV-2 syncytia formation in cells treated with a furin inhibitor compared to untreated cells. (1) Free spike proteins, along with other structural proteins and furin, are trafficked to the plasma membrane through the TGN secretory pathway. (2) Spike S1 subunit binds to ACE2 receptor on neighbouring cell. (3) Furin on host or neighbouring cell cleaves spike protein at S1/S2 site. (4) Additional conformational changes and cleavage events of the spike protein lead to membrane fusion of neighbouring cells and formation of syncytia. Described steps are blocked in cells treated with furin inhibitors. Although, alternative entry mediated by endocytosis and cleavage by cathepsin-L allows for virions to enter and infect healthy cells. Figure created with BioRender.com.

peptide inhibitor, decanoyl-RVKR-chloromethylketone (CMK) (21). CMK binds to the catalytic site of furin present in host cells and blocks it from cleaving the S1/S2 site. CMK not only blocks viral entry, but also suppresses the proteolytic cleavage of spike and thereby, syncytium formation (21). Therefore, furin can serve as an appealing and effective target for host-directed antivirals that inhibit syncytia formation. Admittedly, there are limitations to the use of monotherapy furin inhibitors. Researcher have found that other proteases such as TMPRSS2, MMP2/9 and neutrophil elastase can also cleave at the S1/S2 site (11, 23). Furthermore, SARS-CoV-2 can entry via an alternate route of endocytosis. In the lysosomal

route, cathepsin-L is used to cleave the spike protein triggering virus-endosome membrane fusion occurs (24). Therefore, furin inhibitors alone are not sufficient in blocking cell entry and should ideally be coupled with an RNA polymerase inhibitor.

TMEM16 Inhibitors. Researchers identified multiple drug classes that were effective in blocking syncytia including antipsychotics, antidepressants, and first-generation histamine 1 (H1) receptor antagonists. These drugs are also capable of regulating intracellular Ca^{2+} levels and consequently downregulating TMEM16 activity (9). Cells infected with SARS-CoV-2 and expressing spike on their plasma membrane demonstrated an increase of Ca^{2+} in their cytoplasm and increased activity of TMEM16F, a calcium-activated ion channel and scramblase. These events lead to phosphatidylserine (PS) externalization which is required for membrane fusion and chloride secretion which is hypothesized to play a role in COVID-19 pathogenesis (9). The anthelmintic drug, niclosamide was identified as an effective inhibitor of syncytial formation as infected cells treated with niclosamide were no longer syncytial (9). Mechanistically, nicloasmide acts to suppress TMEM16F/Anoctamin 6, by reducing transient levels of Ca^{2+} in the cytoplasm via interception of the signalling pathway needed for Ca^{2+} release from the ER into the cytoplasm (9). TMEM16F is needed to trigger the unsheathing of the S2 subunit of the spike protein and eventually resulting in membrane fusion (6). It was observed that 1μM of niclosamide was sufficient in reducing the frequency and amplitude of the Ca2+ oscillations (9). The general mechanism of TMEM16-mediated syncytia inhibition is outlined in Figure 2. Many other drugs were identified to successfully

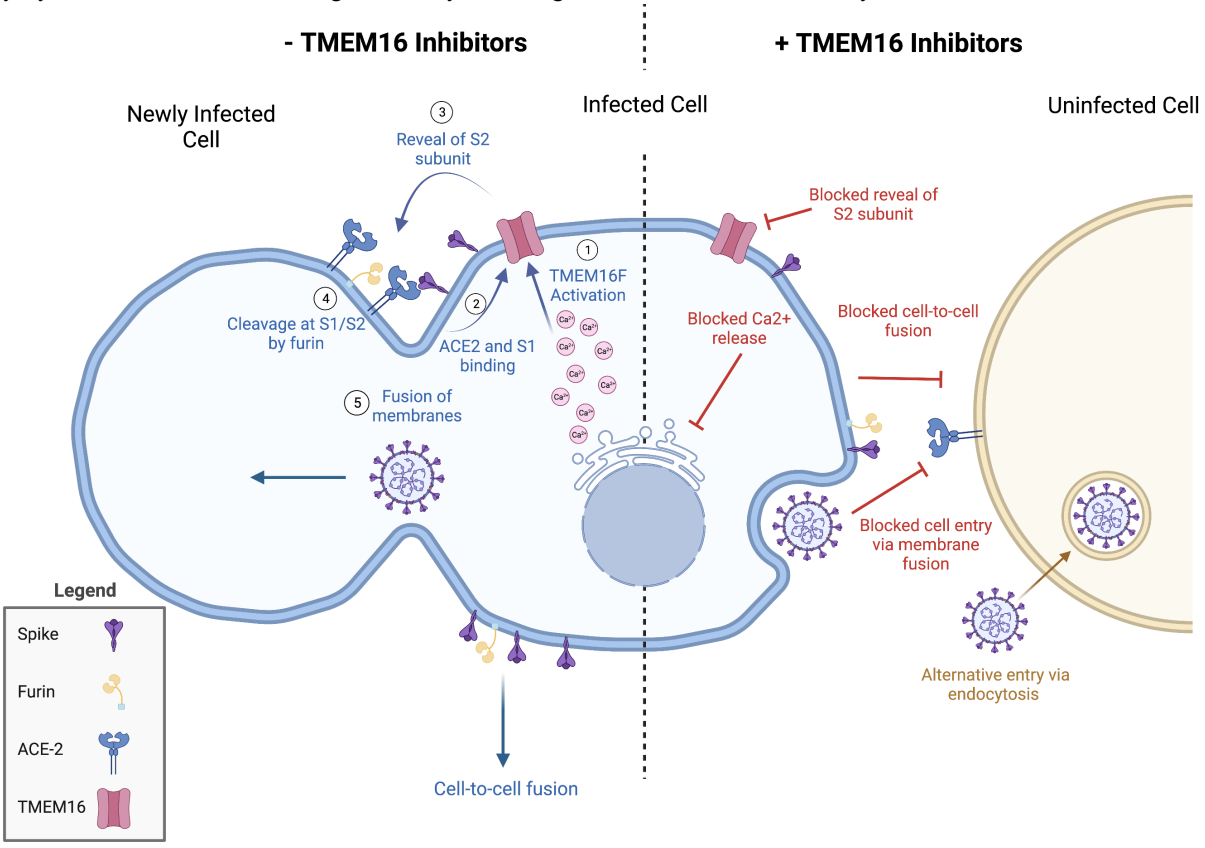

FIG. 2 Overview of SARS-CoV-2 syncytia formation in cells treated with a TMEM16 inhibitor compared to untreated cells. (1) Calcium ions are released from the ER into the cytoplasm of SARS-CoV-2 infected cells to activate calcium dependent TMEM16F located at the plasma membrane. (2) Binding of the S1 spike domain to the ACE-2 receptor on a neighboring cell triggers TMEM16F activity. (3) TMEM16 aids in the shedding of the S1 domain and unsheathing of the S2 subunit with adequate calcium activation. (4) Furin on neighbouring or infected cell cleaves at the S1/S2 site. (5) Additional conformational changes and cleavage events of the spike protein lead to membrane fusion of neighbouring cells and formation of syncytia. Described steps are blocked in cells treated with TMEM16 inhibitors although, alternative entry mediated by endocytosis and cleavage by cathepsin-L allows for virions to enter and infect healthy cells. Figure created with BioRender.com.

inhibit spike-induced syncytia formation such as nitazoxanide, hexachlorophene and dichlorophen (9). The TMEM16 family appears to have an important role in the cell-to-cell fusion of SARS-CoV-2 infected cells. Consequently, there is strong evidence to support the repurposing of TMEM16 antagonists such as niclosamide in drug therapy development to target syncytia formation. Furthermore, these drugs have the potential to act as broad spectrum-acting antivirals as other enveloped viruses may depend on proteins in the TMEM16 family for syncytial formation.

POTENTIAL IMPACT/CONCLUSIONS

Evidently, the primary challenge for studying syncytia morphology is the access to high resolution microscopy for visualization of intra-cellular structures. Although this level of high-resolution microscopy has been present for many years now, such as transmission electron microscopy (TEM), and more recently, cryogenic electron microscopy (cryo-EM), we still do not have an adequate understanding of what viral-induced syncytia look like beyond cell membrane fusion and multi-nucleation. This gap in knowledge highlights the necessity for interdisciplinary collaborations in research. Theoretically, an experimental model that aims to achieve this could use SARS-CoV-2 infected lung organoids and TEM to study the changes in cell morphology, secretion, and transcription before infection and at different time points post-infection. It is compelling to note how the trends in preferred microscopy techniques have changed over the decades. The publication date of many research studies on MGCs coincide with the rise in popularity of TEM in the 1950s to 1970s. Whereas, between 2010 and 2015, cryo-EM was reportedly used in only 29% of soft matter studies that used electron microscopy, despite its invention in the late 1980s (26). However, recently, cryo-EM has grown in popularity within the field of drug design, technology development and molecular virology. Expectantly, we may begin to see cryo-EM deployed in syncytial related research soon. Apart from challenges in bioimaging, current in vitro models modeling spike induced syncytia formation have their limitations as well. Many studies using VeroE6 cells solely expressing the spike protein. Although it creates a simpler model, it drastically simplifies the complex processes that occur and involve other viral proteins, including the SARS-CoV-2 structural proteins, envelope (E) and membrane (M). Future syncytia model should aim to incorporate all structural proteins, for a more comprehensive understanding of syncytia formation.

The urgency of the COVID-19 pandemic has pushed forth major advancements in molecular virology research and vaccine development during what is known as the ‘Coronavirus cascade’ (27). It is estimated that in 2020 alone, almost 200,000 articles were published about the coronavirus pandemic (27). Consequently, some areas of SARS-CoV-2 research was pushed to the forefront while other topics did not receive the same limelight. Although syncytia are evident in severe COVID-19 cases, not enough is known about how they function to produce the lung pathogenicity seen. Therefore, by focusing on establishing a better understanding of the morphology, secretion and transcription of syncytia, there is tremendous potential for the development of novel therapies that target syncytial formation. The development of syncytia inhibitors would not only have a large impact on reducing COVID-19 associated CRDs and lung complications but could also bring forth a broad-spectrum acting antiviral. As discussed, syncytia are a common characteristic among other human disease-causing viruses including respiratory syncytial virus (RSV), human immunodeficiency virus (HIV) and varicella-zoster virus, chicken pox (VZV) (7). An antiviral drug that can inhibit SARS-CoV-2 spike mediated syncytia formation, has the potential to also inhibit syncytia formation in another syncytial-inducing virus especially for host directed antivirals that target proteases used by other viruses, such as the furin-mediated cleavage of the fusion (F) glycoprotein in RSV. Further, viral-induced syncytia formation could play a role in co-infection and increased host susceptibility and virus tropism. Research shows that upon co-infection of RSV and influenza A virus (IAV), hybrid viral particles (HVPs) are produced that can effectively evade IAV-directed antibodies and infect cells lacking IAV receptors (28). The formation of syncytia alongside circulating hybrid viral particles with increased tropism could pose serious threat to individual and public health. More research is needed to determine what role syncytia play in co-infection and susceptibility. Lastly, the impact of this research extends well beyond the scope of virology

and pathogenicity. As discussed, syncytia are important to many natural occurring processes including embryo development, tissue repair and the innate immune response (17, 29, 30). Therefore, an improved understanding of how syncytia function and interact with their environment would also provide new and valuable knowledge on the cellular details of these processes.

ACKNOWLEDGEMENTS

I would like to thank Dr. Jean for his guidance and feedback throughout the research and writing process. His insight and mentorship helped establish the research focus of this article. I would also like to thank my MICB 406 peers for their input and support during discussions which contributed greatly to the development of the final product.

REFERENCES

1. WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int. Retrieved 30 March 2023.
2. Rajah MM, Bernier A, Buchrieser J, Schwartz O. 2022. The Mechanism and Consequences of SARS-CoV-2 Spike-Mediated Fusion and Syncytia Formation. J Mol Biol 434:167280.
3. Edler C, Schröder AS, Aepfelbacher M, Fitzek A, Heinemann A, Heinrich F, Klein A, Langenwalder F, Lütgehetmann M, Meißner K, Püschel K, Schädler J, Steurer S, Mushumba H, Sperhake J-P. 2020. Dying with SARS-CoV-2 infection-an autopsy study of the first consecutive 80 cases in Hamburg, Germany. Int J Legal Med 134:1275–1284.
4. COVID-19 and the Lungs | NHLBI, NIH. https://www.nhlbi.nih.gov/covid/lungs. Retrieved 30 March 2023.
5. Trypsteen W, Cleemput JV, Snippenberg W van, Gerlo S, Vandekerckhove L. 2020. On the whereabouts of SARS-CoV-2 in the human body: A systematic review. PLOS Pathog 16:e1009037.
6. Lin L, Li Q, Wang Y, Shi Y. 2021. Syncytia formation during SARS-CoV-2 lung infection: a disastrous unity to eliminate lymphocytes. 6. Cell Death Differ 28:2019–2021.
7. Leroy H, Han M, Woottum M, Bracq L, Bouchet J, Xie M, Benichou S. 2020. Virus-Mediated Cell-Cell Fusion. Int J Mol Sci 21:9644.
8. Lee DM, Chen EH. 2019. Drosophila Myoblast Fusion: Invasion and Resistance for the Ultimate Union. Annu Rev Genet 53:67–91.
9. Braga L, Ali H, Secco I, Chiavacci E, Neves G, Goldhill D, Penn R, Jimenez-Guardeño JM, Ortega-Prieto AM, Bussani R, Cannatà A, Rizzari G, Collesi C, Schneider E, Arosio D, Shah AM, Barclay WS, Malim MH, Burrone J, Giacca M. 2021. Drugs that inhibit TMEM16 proteins block SARS-CoV-2 spike-induced syncytia. 7861. Nature 594:88–93.
10. Zhang Z, Zheng Y, Niu Z, Zhang B, Wang C, Yao X, Peng H, Franca DN, Wang Y, Zhu Y, Su Y, Tang M, Jiang X, Ren H, He M, Wang Y, Gao L, Zhao P, Shi H, Chen Z, Wang X, Piacentini M, Bian X, Melino G, Liu L, Huang H, Sun Q. 2021. SARS-CoV-2 spike protein dictates syncytium-mediated lymphocyte elimination. 9. Cell Death Differ 28:2765–2777.
11. Benlarbi M, Laroche G, Fink C, Fu K, Mulloy RP, Phan A, Ariana A, Stewart CM, Prévost J, Beaudoin-Bussières G, Daniel R, Bo Y, El Ferri O, Yockell-Lelièvre J, Stanford WL, Giguère PM, Mubareka S, Finzi A, Dekaban GA, Dikeakos JD, Côté M. 2022. Identification and differential usage of a host metalloproteinase entry pathway by SARS-CoV-2 Delta and Omicron. iScience 25:105316.
12. Willett BJ, Grove J, MacLean OA, Wilkie C, De Lorenzo G, Furnon W, Cantoni D, Scott S, Logan N, Ashraf S, Manali M, Szemiel A, Cowton V, Vink E, Harvey WT, Davis C, Asamaphan P, Smollett K, Tong L, Orton R, Hughes J, Holland P, Silva V, Pascall DJ, Puxty K, da Silva Filipe A, Yebra G, Shaaban S, Holden MTG, Pinto RM, Gunson R, Templeton K, Murcia PR, Patel AH, Klenerman P, Dunachie S, Haughney J, Robertson DL, Palmarini M, Ray S, Thomson EC. 2022. SARS-CoV-2 Omicron is an immune escape variant with an altered cell entry pathway. 8. Nat Microbiol 7:1161–1179.
13. Rajah MM, Hubert M, Bishop E, Saunders N, Robinot R, Grzelak L, Planas D, Dufloo J, Gellenoncourt S, Bongers A, Zivaljic M, Planchais C, Guivel-Benhassine F, Porrot F, Mouquet H, Chakrabarti LA, Buchrieser J, Schwartz O. 2021. SARS-CoV-2 Alpha, Beta, and Delta variants display enhanced Spike-mediated syncytia formation. EMBO J 40:e108944.
14. Ohtsu M, Sato Y, Kurihara D, Suzaki T, Kawaguchi M, Maruyama D, Higashiyama T. 2017. Spatiotemporal deep imaging of syncytium induced by the soybean cyst nematode Heterodera glycines. Protoplasma 254:2107–2115.
15. Sutton JS, Weiss L. 1966. TRANSFORMATION OF MONOCYTES IN TISSUE CULTURE INTO MACROPHAGES, EPITHELIOID CELLS, AND MULTINUCLEATED GIANT CELLS : An Electron Microscope Study. J Cell Biol 28:303–332.
16. Tsukamoto Y, Kotani T, Kohama K, Sakuma S, Sasaki F. 1999. Nuclear proliferation in syncytia during avian reovirus replication. Can J Vet Res 63:282–283.

17. Ahmadzadeh K, Vanoppen M, Rose CD, Matthys P, Wouters CH. 2022. Multinucleated Giant Cells: Current Insights in Phenotype, Biological Activities, and Mechanism of Formation. Front Cell Dev Biol 10.
18. Li X, Yuan H, Li X, Wang H. 2023. Spike protein mediated membrane fusion during SARS-CoV-2 infection. J Med Virol 95:e28212.
19. Szakasits D, Heinen P, Wieczorek K, Hofmann J, Wagner F, Kreil DP, Sykacek P, Grundler FMW, Bohlmann H. 2009. The transcriptome of syncytia induced by the cyst nematode Heterodera schachtii in Arabidopsis roots. Plant J 57:771–784.
20. Barreto-Vieira DF, da Silva MAN, de Almeida ALT, Rasinhas A da C, Monteiro ME, Miranda MD, Motta FC, Siqueira MM, Girard-Dias W, Archanjo BS, Bozza PT, L. Souza TM, Gomes Dias SS, Soares VC, Barth OM. 2022. SARS-CoV-2: Ultrastructural Characterization of Morphogenesis in an In Vitro System. Viruses 14:201.
21. Barreto-Vieira DF, da Silva MAN, de Almeida ALT, Rasinhas A da C, Monteiro ME, Miranda MD, Motta FC, Siqueira MM, Girard-Dias W, Archanjo BS, Bozza PT, L. Souza TM, Gomes Dias SS, Soares VC, Barth OM. 2022. SARS-CoV-2: Ultrastructural Characterization of Morphogenesis in an In Vitro System. Viruses 14:201.
22. Cheng Y-W, Chao T-L, Li C-L, Chiu M-F, Kao H-C, Wang S-H, Pang Y-H, Lin C-H, Tsai Y-M, Lee W-H, Tao M-H, Ho T-C, Wu P-Y, Jang L-T, Chen P-J, Chang S-Y, Yeh S-H. 2020. Furin Inhibitors Block SARS-CoV-2 Spike Protein Cleavage to Suppress Virus Production and Cytopathic Effects. Cell Rep 33:108254.
23. Beaudoin CA, Pandurangan AP, Kim SY, Hamaia SW, Huang CL-H, Blundell TL, Vedithi SC, Jackson AP. 2022. In silico analysis of mutations near S1/S2 cleavage site in SARS-CoV-2 spike protein reveals increased propensity of glycosylation in Omicron strain. J Med Virol 94:4181–4192.
24. Mustafa Z, Zhanapiya A, Kalbacher H, Burster T. 2021. Neutrophil Elastase and Proteinase 3 Cleavage Sites Are Adjacent to the Polybasic Sequence within the Proteolytic Sensitive Activation Loop of the SARS-CoV-2 Spike Protein. ACS Omega 6:7181–7185.
25. Jackson CB, Farzan M, Chen B, Choe H. 2022. Mechanisms of SARS-CoV-2 entry into cells. 1. Nat Rev Mol Cell Biol 23:3–20.
26. Franken LE, Grünewald K, Boekema EJ, Stuart MCA. 2020. A Technical Introduction to Transmission Electron Microscopy for Soft-Matter: Imaging, Possibilities, Choices, and Technical Developments. Small 16:1906198.
27. Else H. 2020. How a torrent of COVID science changed research publishing — in seven charts. Nature 588:553–553.
28. Haney J, Vijayakrishnan S, Streetley J, Dee K, Goldfarb DM, Clarke M, Mullin M, Carter SD, Bhella D, Murcia PR. 2022. Coinfection by influenza A virus and respiratory syncytial virus produces hybrid virus particles. 11. Nat Microbiol 7:1879–1890.
29. Ma Z, Sagrillo-Fagundes L, Mok S, Vaillancourt C, Moraes C. 2020. Mechanobiological regulation of placental trophoblast fusion and function through extracellular matrix rigidity. Sci Rep 10:5837.
30. Giordano-Santini R, Linton C, Hilliard MA. 2016. Cell-cell fusion in the nervous system: Alternative mechanisms of development, injury, and repair. Semin Cell Dev Biol 60:146–154.