Pathogenic Human Coronavirus Envelope Protein: A Clear Link to Immunopathology?

Preprint · May 2020

2 authors, including:

Dewald Schoeman
University of the Western Cape
3 PUBLICATIONS **448** CITATIONS

SEE PROFILE

Pathogenic Human Coronavirus Envelope Protein: A Clear Link to Immunopathology?

Dewald Schoeman and Burtram C. Fielding*

Molecular Biology and Virology Research Laboratory, Department of Medical Biosciences, University of the Western Cape, Bellville, Cape Town, 7535, Western Cape, South Africa

*Correspondence: bfielding@uwc.ac.za; Tel.: +27-21-959-2949

Abstract

Since the severe acute respiratory syndrome (SARS) outbreak in 2003, human coronaviruses (hCoVs) have been identified as causative agents of severe acute respiratory tract infections. Two more hCoV outbreaks have since occurred, the most recent being SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19). The clinical presentation of SARS and MERS is remarkably similar to COVID-19, with hyperinflammation causing a severe form of the disease in some patients. Previous studies show that the expression of the SARS-CoV E protein is associated with the hyperinflammatory response that could culminate in acute respiratory distress syndrome (ARDS), a potentially fatal complication. This immune-mediated damage is largely caused by a cytokine storm, which is induced by significantly elevated levels of inflammatory cytokines interleukin (IL)-1β and IL-6, which are partly mediated by the expression of the SARS-CoV E protein. The interaction between the SARS-CoV E protein and the host protein, syntenin, as well as the viroporin function of SARS-CoV E, are linked to this cytokine dysregulation. This review aims to compare the clinical presentation of virulent hCoVs with a specific focus on the cause of the immunopathology. The review also proposes that inhibition of IL-1β and IL-6 in severe cases can improve patient outcome.

Keywords: human coronavirus; SARS-CoV; MERS-CoV; SARS-CoV-2; envelope protein; immunopathology

Introduction

Coronaviruses (CoVs) (order *Nidovirales*) all have positive sense, single-stranded RNA genomes that range in size between 26 and 32 kilobases (kb) [1, 2]. While they predominantly infect animals, some have, in decades past, been able to cross the species barrier and infect humans. Seven human CoVs (hCoVs) have been identified, of which four – hCoVs 229E, NL63, OC43, and HKU1 – are distributed globally, circulating continuously within the human population, causing mild-to-moderate, self-limiting infections [3]. Conversely, the other three hCoVs, – severe acute respiratory syndrome (SARS)-CoV, Middle East respiratory syndrome (MERS)-CoV, and SARS-CoV-2 – are more virulent and have caused deadly outbreaks during the past two decades [4-6].

SARS-CoV caused the first deadly hCoV outbreak in 2003, which was successfully contained in little over six months [7]. The SARS-CoV outbreak resulted in 8096 laboratory-confirmed infections worldwide with 774 deaths, a case-fatality rate of 9.6% [8]. In 2012, the MERS-CoV was identified as the causative agent of MERS in Saudi-Arabia [9]. The MERS-CoV outbreak of 2012 saw a case-fatality rate of 34.4% from 2499 laboratory-confirmed cases and 861 associated deaths as of December 2019 [10]. Then, at the end of 2019, SARS-CoV-2 (formerly known as 2019-nCoV) was reported to be responsible for another outbreak of a SARS-like disease in Wuhan, China [11-13]. As of 20 May 2020, 4 789 205 confirmed cases of SARS-CoV-2 infections with at least 318 789 deaths were reported worldwide [14].

Undoubtedly, SARS-CoV-2 has an infective profile vastly different from that of the SARS-CoV and MERS-CoV. This is especially evident by the incredibly rapid spread, but much lower case-fatality rate of SARS-CoV-2. The disease associated with the virus was named coronavirus disease 2019 (COVID-19) and is the first hCoV outbreak to be declared a pandemic [15, 16]. This review compares the clinical presentation of the virulent hCoVs, SARS-CoV and MERS-CoV, to the symptoms reported in COVID-19 patients to date. Evidence is also presented to call attention to the hCoV protein responsible for the immunopathology often seen in severe cases of pathogenic hCoV infections, and how this protein drives the hyperinflammatory response behind this immunopathology. The major inflammatory cytokines involved in this response are highlighted and linked to the inflammatory cytokines reported in COVID-19 patients. Interim potential treatment options that can minimise disease severity, alleviate the burden of disease, and improve patient outcome are proposed while antiviral and vaccine research is still ongoing.

SARS-CoV and MERS-CoV: a historical perspective

SARS- and MERS-CoV cause more severe disease, even in immunocompetent, healthy individuals [17]. Patients infected with SARS-CoV present with symptoms resembling atypical pneumonia, exhibiting fever, chills, headache, malaise, myalgia, and dry cough [18-20]. Those infected with MERS-CoV report similar non-specific symptoms, but demonstrate a much higher case-fatality rate, particularly for elderly persons and those with underlying medical conditions [21-23]. In some cases, a small proportion of both SARS and MERS patients develop gastrointestinal symptoms (GIT) such as nausea, vomiting, or diarrhoea.

The incubation period for SARS is typically between two and seven days, but can be up to fourteen days, while for MERS it ranges from two to fourteen days with a median of approximately five days [24, 25]. Unlike the four common hCoVs, the severity of SARS and MERS could likely be attributed to their lack of continuous circulation in the human population. The latter hCoVs had not adapted well to humans as hosts and only managed to cause outbreaks after crossing the species barrier, gaining access to the human population from their animal reservoir through an intermediate host [26-28].

Patients infected with SARS-CoV and MERS-CoV are at risk of developing acute respiratory distress syndrome (ARDS), a common complication for both viruses. SARS-CoV and MERS-CoV infections have been linked to diffuse alveolar damage (DAD) and are characterised by increased capillary permeability in the lungs, fluid accumulation in the alveoli, coupled with impaired fluid removal mechanisms that culminate in pulmonary oedema, inefficient gas exchange, and death [29-31]. The incidence of ARDS can be up to 25% in SARS patients, with an associated mortality rate of approximately 50% in these patients [29, 30]. In MERS patients, the incidence of ARDS was less commonly reported, but could develop in 12-20% of patients [22, 32]. In comparison, some studies have reported that 17-41% of COVID-19 patients had developed ARDS [33, 34].

Pro-inflammatory cytokines drive the inflammatory response behind ARDS and are a major contributor to the progression thereof [35]. Several studies report elevated levels of pro-inflammatory cytokines and chemokines (*i.e.* interleukin (IL)-1β, IL-6, IL-8, IL-12, tumour necrosis factor α (TNF-α), interferon γ (IFN-γ), CXCL9, CXCL10, CCL2, CCL3, CCL5, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon-γ inducible

protein 10 kD (IP-10)) associated with the development of ARDS in both SARS and MERS patients [26, 35, 36].

SARS-CoV-2

A lack of epidemiological and serological information on SARS-CoV-2 currently limits our understanding of COVID-19, but patient data from hospitals in Wuhan have provided some insight into its clinical presentation. Patients exhibit fever, dry cough, myalgia, and shortness of breath with ARDS as a common complication [33, 37, 38]. A small number of people also developed GIT symptoms [39, 40]. Similar to SARS and MERS, the elderly and those with underlying, chronic medical conditions, such as diabetes, hypertension, cardiovascular disease, and chronic obstructive pulmonary disease (COPD) are more prone to serious outcomes; complications associated with ARDS and a cytokine storm, often succumbing to the infection [37, 39]. Interestingly, patients who developed ARDS and are admitted to the ICU also have higher levels of inflammatory cytokines, consistent with severe SARS and MERS infections [33, 35, 37, 41, 42].

Like SARS and MERS, these cytokines typically include IL-1, IL-2, IL-4, IL-7, IL-10, IL-12, IL-13, IL-17, granulocyte colony-stimulating factor (GCSF), macrophage colony-stimulating factor (MCSF), IP-10, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1-α (MIP-1α), hepatocyte growth factor (HGF), IFN-γ, and TNF-α and, when released collectively in hyperinflammatory conditions, are referred to as a cytokine storm [37, 43-45]. Already, several reports have remarked on the clinical similarity between COVID-19, MERS and SARS with respect to its clinical presentation [37, 38, 46-56].

The exact cause of this immune-mediated damage, however, remains largely unknown. However, the answer may lie in the mechanics of the viral life cycle and the components that orchestrate it. After all, some viral proteins, especially those involved in pathogenesis, adversely affect the host cell and can be directly implicated in the development of symptoms and, ultimately, the clinical presentation [57].

Viral proteins: at the expense of the host

Viruses by their very nature rely entirely on their host cells for replication, propagation, and, ultimately, survival which is achieved by subverting the protein-protein interaction (PPI) networks of their host cells [58-60]. This subversion requires that viruses encode proteins with the necessary motifs to exploit the network of proteins that govern certain host cell processes of benefit to them [61, 62]. The specific motifs, or stretches of peptide sequences, exploited by viruses have received some attention, but, for the most part, have been quite understudied, despite their importance in viral infections. They are grouped into different categories depending on the purpose of the motif and these motifs are employed by several pathogenic viruses to exploit the host cell pathways that can promote the progression of the viral life cycle [62-64].

About one-third of the 3'-carboxyl terminus of hCoVs genomes encode for structural proteins as well as additional, so-called accessory proteins [65]. While the four structural proteins, spike (S), membrane (M), nucleocapsid (N), and envelope (E), are important for the assembly of a structurally complete virus, the accessory proteins are generally not essential for viral replication *in vitro* [65-68]. While each structural protein has its respective function(s), the E protein is the most enigmatic of them all and is also involved in very important aspects of the coronaviral life cycle. Its involvement in viral assembly is evident by its requirement in the formation of the viral envelope and virus-like particles, while the transmembrane domain (TMD) of E is necessary for the release of viral particles [69, 70]. Of particular relevance to this paper, and the current COVID-19 pandemic, however, is the function of E in the pathogenesis of hCoV infections. Data on the role of E exists predominantly for the prototypic SARS-CoV, which has been studied the most extensively, with some studies for MERS-CoV E.

E protein: a contributor to hCoV pathogenesis

Effective management and patient care of COVID-19 dictates that we have a better understanding of the disease initiation and progression, or pathogenesis. In the case of virulent viruses, it stands to reason that the natural progression of the viral life cycle would adversely affect the host. These adverse effects inherently give rise to symptoms and, ultimately, manifest clinically. Two documented functions of the hCoV E protein contribute to the pathogenesis of severe hCoV infections.

The PDZ-binding motif (PBM)

All CoV E proteins share the same general architecture; a short, hydrophilic amino (N)-terminus, approximately 8-12 residues in length, a subsequent 21-29 residue long hydrophobic region which typically contains two to four cysteine residues, followed by the hydrophilic C-terminus, which accounts for the largest portion of the protein, 39-76 residues in length [65]. The last four residues of the C-terminus consists of a motif that allows binding to the postsynaptic density protein 95 (PSD95)/Drosophila disc large tumour suppressor (Dlg1)/zonula occludens-1 protein (zo-1) (PDZ) domain; a domain found in all eukaryotic host cells that functions as a protein-protein recognition sequence to drive host PPIs of significance to viruses [71]. These PDZ domains are found in a multitude of eukaryotic proteins and bind to a specific peptide sequence usually found at the end of the target protein C-terminus [72, 73]. Some viruses, including SARS-CoV, encode proteins with a PDZ-binding motif (PBM) that enables them to exploit the PDZ domains of these host proteins to their advantage [74, 75]. This strategy is employed by viruses to modulate various cellular processes including cell-cell junctions, cellular polarity, and signal transduction pathways for the purpose of viral replication, dissemination, and pathogenesis [71]. The terminal portion of the SARS-CoV E protein C-terminus contains a PBM that contributes to its viral pathogenesis and is known to interact with five host proteins [76]. It is classified as a type II PBM, characterised by the consensus sequence X-φ-X-φCOOH, where X represents any amino acid and φ is a hydrophobic residue, usually V, I or L [77].

The role of SARS-CoV E in the immune-mediated pathology of severe SARS infections is very well demonstrated by its interaction with the host cell protein, syntenin [78]. Mice infected with recombinant SARS-CoV (rSARS-CoV), containing a fully functional E protein, exhibited lung pathology characterised by severe oedema, areas of profuse haemorrhage, and cellular infiltrates. Further analysis showed that the PBM of SARS-CoV E interacted with the PDZ domain of syntenin and triggered an overexpression of pro-inflammatory cytokines that was mediated by the p38 mitogen-activated protein kinase (MAPK) pathway. Expression of pro-inflammatory cytokines IL-1β and IL-6 as well as the acute phase protein serum amyloid A was notably increased. This resulted in an exacerbated the immune response towards the infection and the characteristic tissue damage and oedema ensued. The infection culminated in ARDS, consistent with severe cases of SARS-CoV infection. Mice infected with rSARS-CoV succumbed to the infection, while all mice infected with rSARS-CoV lacking E (ΔE) survived [78]. Moreover, the authors reported an 80% increase in the survival rate of mice infected with

rSARS-CoV when treated with a p38 MAPK inhibitor. This, notably, demonstrates a clear relationship between the pathogenesis and clinical manifestation of SARS-CoV infections, as a direct consequence of the E protein. It also shows that the mortality rate of infected cases can be markedly reduced by limiting the aberrant immune response with a p38 MAPK inhibitor.

So far, the novelty of SARS-CoV-2 has prohibited its complete characterisation which makes it challenging to confirm whether the functions of its viral proteins do, in fact, coincide with those already established for other hCoVs, like SARS-CoV. Despite its novelty, SARS-CoV-2 shows a remarkable similarity to SARS-CoV in both clinical and genetic features, making it easier to use our existing knowledge of SARS-CoV to understand SARS-CoV-2 better. Previous reports have remarked that the overall sequence similarity of the E protein among hCoVs is poor [79, 80]. Still, comparing the E proteins of the pathogenic hCoVs, SARS-CoV, MERS-CoV, and SARS-CoV-2, shows a very high sequence similarity between SARS-CoV E and SARS-CoV-2 E, confirmed by only one other report and supporting the observed clinical similarity between the two hCoVs [81]. This similarity, however, is not shared with the MERS-CoV E protein.

A sequence comparison of the virulent hCoV E protein sequences demonstrates that important features such as the topological domains, conserved residues, and the PBM also remain largely intact across these hCoVs (Figure 1). The secondary structure of SARS-CoV E shows that it contains one TMD after a short N-terminus and, based on the similarity between SARS-CoV E and SARS-CoV-2 E having only a four amino acid difference, SARS-CoV-2 E follows the same architecture; one TMD that is most likely in the same location and consists of the same residues (Figure 1). Certain key residues are also conserved, particularly the cysteine residues at positions 40, 43, and 43 (C40, C43, C44), and a proline residue at position 54 (P54) (Figure 1). Cysteine residues adjacent to the TMD of integral membrane proteins, like E, serve as targets for palmitoylation [82]. In different CoV E proteins, palmitoylated cysteine residues are important for viral assembly, protein-membrane interaction, and stabilization of the E protein [79, 83]. The importance of residues C40, C43, and C44 is, thus, highlighted by their conservation and proximity to the TMD. A chimeric SARS-CoV E protein showed the importance of P54 in the localisation of E to the Golgi complex as a chimeric E protein with a mutated P54 residue localized to the plasma membrane instead [84]. The conservation of residues C40, C43, C44, and P54 suggest that they might serve similar purposes in SARS-CoV-2 than what they do in SARS-CoV.

The PBM of each hCoV, except MERS-CoV, also consists of at least two definitive hydrophobic residues (V, I, or L), consistent with the consensus sequence for a type II PBM (Figure 1) [77]. Only one of the four PBM residues in the PBM of MERS-CoV E is hydrophobic and another (tryptophan) is slightly more hydrophilic than hydrophobic, based on the Kyte and Doolittle [85] hydropathy table. However, the scarcity of information on hCoV E proteins other than SARS-CoV, makes it difficult to determine the exact reason for this. Nevertheless, the PBMs of SARS-CoV and SARS-CoV-2 are remarkably identical and, given the role of E in SARS-CoV pathogenesis, it supports the similarity in clinical presentation and severity of these two hCoV infections. It also suggests that the SARS-CoV-2 E PBM might interact with syntenin in manner similar to SARS-CoV E. Accordingly, this would allow for treatment strategies and patient care to adopt a more focussed approach as the existing data on the SARS-CoV E PBM and its role in SARS pathogenesis would be most beneficial in mitigating the immunopathology often seen in severe COVID-19 cases. Understandably, this sequence similarity merely suggests the existence of a relationship between the similarity of the SARS-CoV and SARS-CoV-2 E protein PBMs and the clinical presentations of these hCoV infections. Although it certainly is noteworthy, experimental evidence is required to corroborate whether this relationship is merely incidental, or whether it could potentially allude to the clinical manifestation or severity of a particular hCoV infection and whether it might be of therapeutic value in COVID-19 patients.

The SARS-CoV E PBM further contributes to viral pathogenesis by its interaction with the PDZ domain of the protein associated with *Caenorhabditis elegans* lin-7 protein 1 (PALS1) [74]. The binding of SARS-CoV E to PALS1, a protein normally associated with tight junctions, redistributed it from the tight junctions of the lung epithelium to the ER-Golgi intermediate compartment (ERGIC) where E assembles. The authors proposed that the redistribution of PALS1 can progressively disrupt tight junctions and contribute to the desquamation of the alveolar wall, creating a breach in the epithelial barrier. This would allow virions to infiltrate the underlying tissues and reach the systemic circulation, disseminating to other organs. Although the study only managed to demonstrate the E-mediated redistribution of PALS1 *in vitro*, the clinical importance of this interaction is consistent with histopathological observations made in lung biopsies obtained from SARS-CoV-infected patients and cynomolgus macaques. The biopsies consistently demonstrated that severe DAD to the lung was accompanied by a massive infiltration of monocytes and macrophages in the alveolar space, a thickened epithelial wall, fused alveolar septa, and haemorrhagic septa with necrotic lesions

[19, 86, 87]. Further corroboration comes from studies that show massive recruitment of leukocytes to the site of infection through chemokines and cytokines produced by human airway epithelia, strongly implicating inflammation in the contribution of DAD [35, 88, 89].

Granted, although this interaction has only been demonstrated in SARS-CoV, it should not diminish the possibility of it occurring in a similar fashion in other virulent hCoV infections such as SARS-CoV-2. It is likely that the PBM of SARS-CoV-2 E can also interact with PALS1 in an analogous manner and cause dissemination of the virus. In fact, the presence of a PBM at the C-terminus of each virulent hCoV indicates that they might all be capable of interacting with host proteins, such as syntenin and PALS1, similar to SARS-CoV. Experimental evidence is, of course, warranted to provide a solid scientific basis, but it would also provide much need valuable insight into why hCoVs clinically manifest in different severities.

Viroporin and the inflammasome

The hydrophobic TMD of the E protein is an important component necessary for the assembly of a multimeric structure known as a viroporin; low-molecular-weight proteins that typically contain an amphipathic α-helix and are encoded by many animal viruses. Viroporins oligomerise and can channel various ions, altering the permeability properties of membranes within the host cell. Upon oligomerisation, viroporins form a hydrophilic pore that permits the transport of ions across the membrane as the hydrophilic residues face the interior of the pore and the hydrophobic residues face outward towards the phospholipid bilayer [90, 91]. The SARS-CoV E protein viroporin possesses ion-channel (IC) activity and can transport various ions (Na+, K+, Cl-, and Ca2+) [92, 93]. The importance of this IC property is evident in its contribution to the pathogenesis observed in a SARS infection.

The (NOD)-like receptor protein 3 (NLRP3) inflammasome is a multimeric molecular platform that can be activated by several factors, including increased levels of intracellular Ca2+, and contributes to the inflammatory response by stimulating IL-1β production [94, 95]. The IC activity of the SARS-CoV E protein has been linked to activation of the inflammasome and disease severity [96]. Mice infected with IC-proficient rSARS-CoV E developed pulmonary oedema, lung damage, and succumbed to the infection due to significantly increased levels of inflammatory cytokines IL-1β, IL-6, and TNF-α. Conversely, mice infected with IC-deficient rSARS-CoV E exhibit reduced levels of inflammasome-activated IL-1β, and mice recovered from the infection. The IC activity of SARS-CoV E, therefore, directly correlates with inflammasome activation and an ensuing inflammatory response that causes lung damage. The

inflammatory pathology was attributed to a Ca2+ imbalance that activated the NLRP3 inflammasome and induced the production of IL-1β [92]. Only two other hCoV E proteins have been shown to possess IC activity: MERS-CoV and hCoV-229E [97, 98]. However, since no experimental evidence exists to link the IC property of either E protein to NLRP3 inflammasome activation, it can only be hypothesised as to whether these hCoVs are equally capable of inducing a pathologic immune response as SARS-CoV does.

Several other pathogenic viruses also possess viroporin proteins capable of activating the NLRP3 inflammasome; the small hydrophobic (SH) protein of respiratory syncytial virus, influenza virus M2 protein, encephalomyocarditis virus 2B protein, rhinovirus 2B protein, and the hepatitis C virus (HCV) p7 protein [91]. It is also worth mentioning that a number of viroporin inhibitors have been researched in an effort to inhibit the IC properties of the picornavirus, HCV, SARS-CoV, HIV-1, and influenza A virus. Most inhibitors, however, have exhibited some challenges, including mere moderate inhibition, the formation of resistant variants of viruses, and cytotoxic concentrations, preventing the clinical implementation of such inhibitors [99]. Given the challenges faced with these inhibitors, perhaps it would be more prudent to divert the attention towards addressing the fundamental source of viroporins: the viral protein itself.

Cytokines IL-1β and IL-6 in SARS and COVID-19 immunopathology

The presence of IL-1β in the pathogenesis and immunopathology of SARS has been well-demonstrated. Interleukin-1β is a potent inflammatory cytokine – the result of a series of cellular signals and stimuli, involving the nuclear factor kappa B (NF-κB) pathway and the NLRP3 inflammasome [95]. A variety of stimuli is capable of inducing IL-1β production, including products of infectious agents, ionic imbalances inside the cell, exogenous particulates, and molecules associated with cellular damage [95]. Once released into circulation, IL-1β can cause inflammation and perpetuate the inflammatory response by inducing IL-6 production [100-102]. Mice deficient in IL-1β displayed no levels of circulating IL-6 in response to turpentine [103]. Interleukin-1β can also modulate the production of IL-6 through STAT3 and NF-κB-dependent signalling pathways and involves acute phase proteins produced by the liver [104]. This demonstrates that the NF-κB pathway is quite involved in the production of

inflammatory cytokines and that targeting this pathway could be of therapeutic benefit at multiple levels: IL-1β production, IL-6 production, and IL-1β-induced IL-6 production.

Moreover, mice infected with IC-deficient rSARS-CoV E exhibited reduced levels of inflammasome-activated IL-1β in their lungs [96]. This reduction in IL-1β was accompanied by reduced levels of TNF and IL-6, demonstrating the importance of the E protein in the induction of an aberrant inflammatory response in SARS-CoV mice that contributes to the development of a cytokine storm, and ultimately culminates in ARDS.

Discussion and Conclusion

Despite the importance of the hCoV E protein, it is still poorly characterised and quite understudied. And although much progress has been made in hCoV research, the novelty of SARS-CoV-2 clearly leaves much still to be answered. The sequence similarity between the E proteins of SARS-CoV and SARS-CoV-2 strongly suggest the likelihood that these two proteins serve nearly identical purposes in the pathogenesis of COVID-19. Admittedly, a great divergence exists in the amino acid sequences of the E protein between the different CoV groups and, to an extent, within some of the groups. But the overall features and functions of the CoV E still remain largely intact [65]. The importance of the E protein is evident by its involvement in the pathogenesis of SARS-CoV, and possibly SARS-CoV-2, making it an ideal therapeutic candidate. Already, a p38 MAPK inhibitor has shown promise in mice by alleviating the inflammation-induced symptoms brought on by the SARS-CoV E protein. Given the involvement of hCoV E in various aspects of the coronaviral life cycle, targeting E could hold the potential to stopping the spread of infection while simultaneously alleviating the symptoms and managing complications such as ARDS in severe SARS-CoV infections. Coronaviral research would certainly benefit from investigating the therapeutic potential of a p38 MAPK inhibitor in a SARS-CoV-2 infection of mice. The gravity of the COVID-19 pandemic warrants more research into hCoVs and how such outbreaks can be addressed, now more than ever.

Currently, vaccine and antiviral research are being done at a near-unprecedented rate, but while an effective countermeasure might only be available in as soon as twelve months, the hCoV pandemic continues to have a significant impact on people all over the world. The SARS-CoV

E protein is paramount to the pathogenesis of the SARS disease as rSARS-CoV-ΔE viruses show no excessive inflammatory response and spare mice from immune-mediated lung damage. Our paper proposes the use of immunomodulatory or anti-inflammatory drugs that specifically target the already well-characterised inflammatory pathways activated by SARS-CoV E. Given the importance of IL-1β and IL-6 in the development of ARDS, drugs that expressly target IL-1β and IL-6 could lead to more favourable patient outcomes and reduce the rising mortality rate of COVID-19 while vaccine and antiviral research continue.

Amid the global rise in the mortality rate of COVID-19, effective management of inflammation and the cytokine storm, the crucial features of ARDS, should be of considerable priority. The use of the IL-6 receptor blocker, tocilizumab effectively reversed the cytokine storm in acute lymphocytic anaemia [105, 106]. Tocilizumab has, accordingly, been suggested for use in the treatment of severe COVID-19, where Xu, et al. [107] has reported some promise in severe COVID-19 patients [108, 109]. Already, blocking IL-1β activity in a broad array of inflammatory diseases has shown reduced disease severity and a reduction in the burden of disease [110]. Inhibitors of IL-1 typically include the IL-1 receptor antagonist (Anakinra), the soluble decoy receptor (Rilonacept), and the anti-IL-1β monoclonal antibody (Canakinumab) [111]. The efficacy of rilonacept and canakinumab has even garnered approval by pharmaceutical companies, making such IL-1-directed therapies deserving of study as potential treatments to manage severe cases of COVID-19 [112].

The cellular pathways that lead to IL-1β and IL-6 production are well-characterised and could also serve as valuable therapeutic targets. A p38 MAPK pathway inhibitor led to an 80% survival rate of rSARS-CoV-infected mice, showing both the relevance of this pathway in SARS infections and the potential of this inhibitor in successfully managing severe cases of COVID-19 [78]. Furthermore, inhibition of the Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway by ruxolitinib is effective in the treatment of haemophagocytic lymphohistiocytosis, a hyperinflammatory condition also characterised by a cytokine storm [113]. The JAK-STAT pathway is a common signal transduction pathway involved in the expression of many other cytokines also responsible for the immune-mediated damage of ARDS typical of severe SARS cases. Accordingly, this pathway can also be a target for blocking multiple cytokines simultaneously.

The importance of the hCoV E protein and its associated pathways is also demonstrated in the potential of a SARS-CoV-2 vaccine that lacks an E protein. Without the E protein to induce a cytokine storm, and subsequent complications like ARDS, undesired side-effects will be limited, while the vaccine still confers the necessary protection. Some studies have already demonstrated the potential of developing rSARS-CoV-ΔE vaccines, or ones with a mutated E protein to limit pathogenesis while still conferring the needed protection against a viral challenge after vaccination [114-116]. Vaccines based on rSARS-CoV-ΔE remain their immunogenicity and efficacy, developing robust cellular and humoral immune responses and effective despite an impaired ability to replicate in the host. One study even showed that a rSARS-CoV-ΔE-based vaccine can protect both young and aged mice, with no clinical disease observed in mice of any ages [117]. The authors, however, cautioned prudence in the design of such vaccines, highlighting the need to possibly introduce additional mutations to enhance safety due to the recombinatory nature of CoVs [26, 65].

Admittedly, data on CoV E is sparse, but it should not reflect negatively on the importance of the protein in hCoV infections, especially not in the case of serious ones such as SARS-CoV-2. On the contrary, the importance of the E protein should, instead, underpin the need for more research in an effort to limit any likelihood of a future outbreak, possibly a more severe one. If there is anything to learn from the SARS, MERS and COVID-19 outbreaks, it is that we do not know when they will happen nor what the nature of the outbreak will be. The more data we have on previous outbreaks, the better prepared we can be.

Acknowledgments: The authors apologize to any author whose work has been inadvertently omitted from this review article.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Author Contributions: Conceptualization: BCF; original draft preparation: DS; review and editing: DS and BCF

Funding: Burtram C. Fielding receives funding from the National Research Foundation (NRF) (South Africa). Any opinion, findings and conclusions or recommendations expressed in this

material are those of the authors and, therefore, the NRF does not accept any liability in regard thereto.

References

1. Corman, V. M.; Muth, D.; Niemeyer, D.; Drosten, C., Hosts and sources of endemic human coronaviruses. In *Adv. Virus Res.*, Elsevier: 2018; Vol. 100, pp 163-188.

2. Gorbalenya, A. E.; Enjuanes, L.; Ziebuhr, J.; Snijder, E. J., Nidovirales: evolving the largest RNA virus genome. *Virus Res.* **2006,** 117, (1), 17-37.

3. Su, S.; Wong, G.; Shi, W.; Liu, J.; Lai, A. C.; Zhou, J.; Liu, W.; Bi, Y.; Gao, G. F., Epidemiology, genetic recombination, and pathogenesis of coronaviruses. *Trends Microbiol.* **2016,** 24, (6), 490-502.

4. Chafekar, A.; Fielding, B. C., MERS-CoV: understanding the latest human coronavirus threat. *Viruses* **2018,** 10, (2), 93.

5. Kahn, J. S.; McIntosh, K., History and recent advances in coronavirus discovery. *The Pediatric infectious disease journal* **2005,** 24, (11), S223-S227.

6. Pyrc, K.; Berkhout, B.; Van Der Hoek, L., Identification of new human coronaviruses. *Expert Rev. Anti Infect. Ther.* **2007,** 5, (2), 245-253.

7. Hewings-Martin, Y. How do SARS and MERS compare with COVID-19? https://www.medicalnewstoday.com/articles/how-do-sars-and-mers-compare-with-covid-19 (24 April 2020),

8. WHO Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. http://www.who.int/csr/sars/country/table2004_04_21/en/index.html (2 February 2020),

9. Broadbent, L. Coronaviruses – a brief history. https://theconversation.com/coronaviruses-a-brief-history-135506 (24 April 2020),

10. WHO Middle East Respiratory Syndrome, MERS situation update, December 2019. http://www.emro.who.int/health-topics/mers-cov/mers-outbreaks.html (2 February 2020),

11. CDC Human Coronavirus Types. https://www.cdc.gov/coronavirus/types.html (02 February 2020),

12. Gralinski, L. E.; Menachery, V. D., Return of the Coronavirus: 2019-nCoV. *Viruses* **2020,** 12, (2), 135.

13. Kahn, N., New Virus Discovered by Chinese Scientists Investigating Pneumonia Outbreak. *Wall Street Journal* 2020.

14. WHO Coronavirus disease (COVID-2019) situation reports. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200520-covid-19-sitrep-121.pdf?sfvrsn=c4be2ec6_2 (21 May 2020),

15. WHO Naming the coronavirus disease (COVID-19) and the virus that causes it. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it (18 Apirl 2020),

16. WHO WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 (18 April 2020),

17. Avendano, M.; Derkach, P.; Swan, S., Clinical course and management of SARS in health care workers in Toronto: a case series. *CMAJ* **2003,** 168, (13), 1649-1660.

18. Lee, N.; Hui, D.; Wu, A.; Chan, P.; Cameron, P.; Joynt, G. M.; Ahuja, A.; Yung, M. Y.; Leung, C.; To, K., A major outbreak of severe acute respiratory syndrome in Hong Kong. *N. Engl. J. Med.* **2003,** 348, (20), 1986-1994.

19. Peiris, J.; Lai, S.; Poon, L.; Guan, Y.; Yam, L.; Lim, W.; Nicholls, J.; Yee, W.; Yan, W.; Cheung, M., Coronavirus as a possible cause of severe acute respiratory syndrome. *The Lancet* **2003,** 361, (9366), 1319-1325.

20. Peiris, J. S. M.; Chu, C.-M.; Cheng, V. C.-C.; Chan, K.; Hung, I.; Poon, L. L.; Law, K.-I.; Tang, B.; Hon, T.; Chan, C., Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *The Lancet* **2003,** 361, (9371), 1767-1772.

21. Assiri, A.; Al-Tawfiq, J. A.; Al-Rabeeah, A. A.; Al-Rabiah, F. A.; Al-Hajjar, S.; Al-Barrak, A.; Flemban, H.; Al-Nassir, W. N.; Balkhy, H. H.; Al-Hakeem, R. F., Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *The Lancet infectious diseases* **2013,** 13, (9), 752-761.

22. Assiri, A.; McGeer, A.; Perl, T. M.; Price, C. S.; Al Rabeeah, A. A.; Cummings, D. A.; Alabdullatif, Z. N.; Assad, M.; Almulhim, A.; Makhdoom, H., Hospital outbreak of Middle East respiratory syndrome coronavirus. *N. Engl. J. Med.* **2013,** 369, (5), 407-416.

23. Saad, M.; Omrani, A. S.; Baig, K.; Bahloul, A.; Elzein, F.; Matin, M. A.; Selim, M. A.; Al Mutairi, M.; Al Nakhli, D.; Al Aidaroos, A. Y., Clinical aspects and outcomes of 70 patients with Middle East respiratory syndrome coronavirus infection: a single-center experience in Saudi Arabia. *Int. J. Infect. Dis.* **2014,** 29, 301-306.

24. CDC Frequently Asked Questions About SARS. https://www.cdc.gov/sars/about/faq.html (21 April 2020),

25. CDC MERS Clinical Features. https://www.cdc.gov/coronavirus/mers/clinical-features.html# (21 April 2020),

26. Perlman, S.; Netland, J., Coronaviruses post-SARS: update on replication and pathogenesis. *Nature reviews microbiology* **2009,** 7, (6), 439-450.

27. Reusken, C. B.; Haagmans, B. L.; Müller, M. A.; Gutierrez, C.; Godeke, G.-J.; Meyer, B.; Muth, D.; Raj, V. S.; Smits-De Vries, L.; Corman, V. M., Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. *The Lancet infectious diseases* **2013,** 13, (10), 859-866.

28. de Wit, E.; van Doremalen, N.; Falzarano, D.; Munster, V. J., SARS and MERS: recent insights into emerging coronaviruses. *Nature Reviews Microbiology* **2016,** 14, (8), 523.

29. Fowler, R. A.; Lapinsky, S. E.; Hallett, D.; Detsky, A. S.; Sibbald, W. J.; Slutsky, A. S.; Stewart, T. E.; Group, f. t. T. S. C. C., Critically Ill Patients With Severe Acute Respiratory Syndrome. *JAMA* **2003,** 290, (3), 367-373.

30. Lew, T. W. K.; Kwek, T.-K.; Tai, D.; Earnest, A.; Loo, S.; Singh, K.; Kwan, K. M.; Chan, Y.; Yim, C. F.; Bek, S. L.; Kor, A. C.; Yap, W. S.; Chelliah, Y. R.; Lai, Y. C.; Goh, S.-K., Acute Respiratory Distress Syndrome in Critically Ill Patients With Severe Acute Respiratory Syndrome. *JAMA* **2003,** 290, (3), 374-380.

31. Ng, D. L.; Al Hosani, F.; Keating, M. K.; Gerber, S. I.; Jones, T. L.; Metcalfe, M. G.; Tong, S.; Tao, Y.; Alami, N. N.; Haynes, L. M., Clinicopathologic, immunohistochemical, and ultrastructural findings of a fatal case of Middle East respiratory syndrome coronavirus infection in the United Arab Emirates, April 2014. *The American journal of pathology* **2016,** 186, (3), 652-658.

32. Group, W. M.-C. R., State of knowledge and data gaps of Middle East respiratory syndrome coronavirus (MERS-CoV) in humans. *PLoS currents* **2013,** 5.

33. Chen, N.; Zhou, M.; Dong, X.; Qu, J.; Gong, F.; Han, Y.; Qiu, Y.; Wang, J.; Liu, Y.; Wei, Y., Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet* **2020,** 395, (10223), 507-513.

34. Wu, C.; Chen, X.; Cai, Y.; Zhou, X.; Xu, S.; Huang, H.; Zhang, L.; Zhou, X.; Du, C.; Zhang, Y., Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA internal medicine* **2020**.

35. Channappanavar, R.; Perlman, S. In *Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology*, Semin. Immunopathol., 2017; Springer: 2017; pp 529-539.

36. Totura, A. L.; Baric, R. S., SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon. *Curr. Opin. Virol.* **2012,** 2, (3), 264-275.

37. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet* **2020,** 395, (10223), 497-506.

38. Wang, D.; Hu, B.; Hu, C.; Zhu, F.; Liu, X.; Zhang, J.; Wang, B.; Xiang, H.; Cheng, Z.; Xiong, Y., Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA* **2020**.

39. Guan, W.-j.; Ni, Z.-y.; Hu, Y.; Liang, W.-h.; Ou, C.-q.; He, J.-x.; Liu, L.; Shan, H.; Lei, C.-l.; Hui, D. S., Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* **2020**.

40. Guo, Y.-R.; Cao, Q.-D.; Hong, Z.-S.; Tan, Y.-Y.; Chen, S.-D.; Jin, H.-J.; Tan, K.-S.; Wang, D.-Y.; Yan, Y., The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. *Military Medical Research* **2020,** 7, (1), 1-10.

41. Mahallawi, W. H.; Khabour, O. F.; Zhang, Q.; Makhdoum, H. M.; Suliman, B. A., MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. *Cytokine* **2018,** 104, 8-13.

42. Lau, S. K.; Lau, C. C.; Chan, K.-H.; Li, C. P.; Chen, H.; Jin, D.-Y.; Chan, J. F.; Woo, P. C.; Yuen, K.-Y., Delayed induction of proinflammatory cytokines and suppression of innate antiviral response by the novel Middle East respiratory syndrome coronavirus: implications for pathogenesis and treatment. *J. Gen. Virol.* **2013,** 94, (12), 2679-2690.

43. Chen, C.; Zhang, X.; Ju, Z.; He, W., Advances in the research of cytokine storm mechanism induced by Corona Virus Disease 2019 and the corresponding immunotherapies. *Chinese journal of burns* **2020,** 36, E005-E005.

44. Liu, Y.; Zhang, C.; Huang, F.; Yang, Y.; Wang, F.; Yuan, J.; Zhang, Z.; Qin, Y.; Li, X.; Zhao, D., 2019-novel coronavirus (2019-nCoV) infections trigger an exaggerated cytokine response aggravating lung injury. **2020**.

45. Zhou, D.; Dai, S.-M.; Tong, Q., COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. *J. Antimicrob. Chemother.* **2020**.

46. Chen, T.; Wu, D.; Chen, H.; Yan, W.; Yang, D.; Chen, G.; Ma, K.; Xu, D.; Yu, H.; Wang, H., Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ* **2020,** 368.

47. Rasmussen, S. A.; Smulian, J. C.; Lednicky, J. A.; Wen, T. S.; Jamieson, D. J., Coronavirus Disease 2019 (COVID-19) and Pregnancy: What obstetricians need to know. *Am. J. Obstet. Gynecol.* **2020**.

48. Chan, J. F.-W.; Yuan, S.; Kok, K.-H.; To, K. K.-W.; Chu, H.; Yang, J.; Xing, F.; Liu, J.; Yip, C. C.-Y.; Poon, R. W.-S., A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *The Lancet* **2020,** 395, (10223), 514-523.

49. Wang, Y.; Wang, Y.; Chen, Y.; Qin, Q., Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. *J. Med. Virol.* **2020**.

50. Zhang, W.; Zhao, Y.; Zhang, F.; Wang, Q.; Li, T.; Liu, Z.; Wang, J.; Qin, Y.; Zhang, X.; Yan, X., The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The experience of clinical immunologists from China. *Clin. Immunol.* **2020**, 108393.

51. Prompetchara, E.; Ketloy, C.; Palaga, T., Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. *Asian Pac. J. Allergy Immunol.* **2020,** 38, (1), 1-9.

52. Xie, M.; Chen, Q., Insight into 2019 novel coronavirus—an updated intrim review and lessons from SARS-CoV and MERS-CoV. *Int. J. Infect. Dis.* **2020**.

53. Chen, G.; Wu, D.; Guo, W.; Cao, Y.; Huang, D.; Wang, H.; Wang, T.; Zhang, X.; Chen, H.; Yu, H., Clinical and immunologic features in severe and moderate forms of Coronavirus Disease 2019. *medRxiv* **2020**.

54. Chhikara, B. S.; Rathi, B.; Singh, J.; Poonam, F., Corona virus SARS-CoV-2 disease COVID-19: Infection, prevention and clinical advances of the prospective chemical drug therapeutics. *Chemical Biology Letters* **2020,** 7, (1), 63-72.

55. Giwa, A.; Desai, A.; Duca, A., Novel 2019 coronavirus SARS-CoV-2 (COVID-19): an overview for emergency clinicians. *Pediatric Emergency Medicine Practice* **2020,** 17, (5), 1-24.

56. Lin, W.; Wen, J.; Chen, G., Epidemiological and clinical characteristics of SARS-CoV-2 and SARS-CoV: a system review. *medRxiv* **2020**.

57. Manjarrez-Zavala, M. E.; Rosete-Olvera, D. P.; Gutiérrez-González, L. H.; Ocadiz-Delgado, R.; Cabello-Gutiérrez, C., Pathogenesis of viral respiratory infection. *Respiratory Disease and Infection: A New Insight* **2013**, 1.

58. Guth, C. A.; Sodroski, J., Contribution of PDZD8 to stabilization of the human immunodeficiency virus type 1 capsid. *J. Virol.* **2014,** 88, (9), 4612-4623.

59. Gladue, D.; O'Donnell, V.; Baker-Branstetter, R.; Holinka, L.; Pacheco, J.; Fernandez-Sainz, I.; Lu, Z.; Brocchi, E.; Baxt, B.; Piccone, M., Foot-and-mouth disease virus nonstructural protein 2C interacts with Beclin1, modulating virus replication. *J. Virol.* **2012,** 86, (22), 12080-12090.

60. Brito, A. F.; Pinney, J. W., Protein-protein interactions in virus-host systems. *Front. Microbiol.* **2017,** 8, 1557.

61. Zheng, L.-L.; Li, C.; Ping, J.; Zhou, Y.; Li, Y.; Hao, P., The domain landscape of virus-host interactomes. *BioMed research international* **2014,** 2014.

62. Davey, N. E.; Travé, G.; Gibson, T. J., How viruses hijack cell regulation. *Trends Biochem. Sci.* **2011,** 36, (3), 159-169.

63. Gould, C. M.; Diella, F.; Via, A.; Puntervoll, P.; Gemünd, C.; Chabanis-Davidson, S.; Michael, S.; Sayadi, A.; Bryne, J. C.; Chica, C., ELM: the status of the 2010 eukaryotic linear motif resource. *Nucleic Acids Res.* **2010,** 38, (suppl_1), D167-D180.

64. Sobhy, H., A review of functional motifs utilized by viruses. *Proteomes* **2016,** 4, (1), 3.

65. Masters, P. S., The molecular biology of coronaviruses. *Adv. Virus Res.* **2006,** 66, 193-292.

66. Mortola, E.; Roy, P., Efficient assembly and release of SARS coronavirus-like particles by a heterologous expression system. *FEBS Lett.* **2004,** 576, (1-2), 174-178.

67. Wang, C.; Zheng, X.; Gai, W.; Zhao, Y.; Wang, H.; Wang, H.; Feng, N.; Chi, H.; Qiu, B.; Li, N., MERS-CoV virus-like particles produced in insect cells induce specific humoural and cellular imminity in rhesus macaques. *Oncotarget* **2017,** 8, (8), 12686.

68. Liu, D. X.; Fung, T. S.; Chong, K. K.-L.; Shukla, A.; Hilgenfeld, R., Accessory proteins of SARS-CoV and other coronaviruses. *Antiviral Res.* **2014,** 109, 97-109.

69. Corse, E.; Machamer, C. E., The cytoplasmic tails of infectious bronchitis virus E and M proteins mediate their interaction. *Virology* **2003,** 312, (1), 25-34.

70. Ruch, T. R.; Machamer, C. E., The hydrophobic domain of infectious bronchitis virus E protein alters the host secretory pathway and is important for release of infectious virus. *J. Virol.* **2011,** 85, (2), 675-685.

71. Javier, R. T.; Rice, A. P., Emerging theme: cellular PDZ proteins as common targets of pathogenic viruses. *J. Virol.* **2011,** 85, (22), 11544-11556.

72. Gerek, Z. N.; Keskin, O.; Ozkan, S. B., Identification of specificity and promiscuity of PDZ domain interactions through their dynamic behavior. *Proteins: Structure, Function, and Bioinformatics* **2009,** 77, (4), 796-811.

73. Hung, A. Y.; Sheng, M., PDZ domains: structural modules for protein complex assembly. *J. Biol. Chem.* **2002,** 277, (8), 5699-5702.

74. Teoh, K.-T.; Siu, Y.-L.; Chan, W.-L.; Schlüter, M. A.; Liu, C.-J.; Peiris, J. M.; Bruzzone, R.; Margolis, B.; Nal, B., The SARS coronavirus E protein interacts with PALS1 and alters tight junction formation and epithelial morphogenesis. *Mol. Biol. Cell* **2010,** 21, (22), 3838-3852.

75. Castaño-Rodriguez, C.; Honrubia, J. M.; Gutiérrez-Álvarez, J.; DeDiego, M. L.; Nieto-Torres, J. L.; Jimenez-Guardeño, J. M.; Regla-Nava, J. A.; Fernandez-Delgado, R.; Verdia-Báguena, C.; Queralt-Martín, M., Role of severe acute respiratory syndrome

Coronavirus Viroporins E, 3a, and 8a in replication and pathogenesis. *mBio* **2018,** 9, (3), e02325-17.

76. Schoeman, D.; Fielding, B. C., Coronavirus envelope protein: current knowledge. *Virol J.* **2019,** 16, (1), 69.

77. Harris, B. Z.; Lim, W. A., Mechanism and role of PDZ domains in signaling complex assembly. *J. Cell Sci.* **2001,** 114, (18), 3219-3231.

78. Jimenez-Guardeño, J. M.; Nieto-Torres, J. L.; DeDiego, M. L.; Regla-Nava, J. A.; Fernandez-Delgado, R.; Castaño-Rodriguez, C.; Enjuanes, L., The PDZ-binding motif of severe acute respiratory syndrome coronavirus envelope protein is a determinant of viral pathogenesis. *PLoS Pathog.* **2014,** 10, (8), e1004320.

79. Lopez, L. A.; Riffle, A. J.; Pike, S. L.; Gardner, D.; Hogue, B. G., Importance of conserved cysteine residues in the coronavirus envelope protein. *J. Virol.* **2008,** 82, (6), 3000-3010.

80. Ye, Y.; Hogue, B. G., Role of the coronavirus E viroporin protein transmembrane domain in virus assembly. *J. Virol.* **2007,** 81, (7), 3597-3607.

81. Grifoni, A.; Sidney, J.; Zhang, Y.; Scheuermann, R. H.; Peters, B.; Sette, A., A Sequence Homology and Bioinformatic Approach Can Predict Candidate Targets for Immune Responses to SARS-CoV-2. *Cell Host & Microbe* **2020,** 27, (4), 671-680.e2.

82. He, M.; Jenkins, P.; Bennett, V., Cysteine 70 of ankyrin-G is S-palmitoylated and is required for function of ankyrin-G in membrane domain assembly. *J. Biol. Chem.* **2012,** 287, (52), jbc. M112. 417501.

83. Boscarino, J. A.; Logan, H. L.; Lacny, J. J.; Gallagher, T. M., Envelope protein palmitoylations are crucial for murine coronavirus assembly. *J. Virol.* **2008,** 82, (6), 2989-2999.

84. Cohen, J. R.; Lin, L. D.; Machamer, C. E., Identification of a Golgi targeting signal in the cytoplasmic tail of the severe acute respiratory syndrome coronavirus envelope protein. *J. Virol.* **2011,** 85, (12), 5794-5803.

85. Kyte, J.; Doolittle, R. F., A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **1982,** 157, (1), 105-132.

86. Kuiken, T.; Fouchier, R. A.; Schutten, M.; Rimmelzwaan, G. F.; Van Amerongen, G.; van Riel, D.; Laman, J. D.; de Jong, T.; van Doornum, G.; Lim, W., Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *The Lancet* **2003,** 362, (9380), 263-270.

87. Li, B.-j.; Tang, Q.; Cheng, D.; Qin, C.; Xie, F. Y.; Wei, Q.; Xu, J.; Liu, Y.; Zheng, B.-j.; Woodle, M. C., Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. *Nat. Med.* **2005,** 11, (9), 944-951.

88. Lau, Y. L.; Peiris, J. M., Pathogenesis of severe acute respiratory syndrome. *Curr. Opin. Immunol.* **2005,** 17, (4), 404-410.

89. Thiel, V.; Weber, F., Interferon and cytokine responses to SARS-coronavirus infection. *Cytokine Growth Factor Rev.* **2008,** 19, (2), 121-132.

90. Gonzalez, M. E.; Carrasco, L., Viroporins. *FEBS Lett.* **2003,** 552, (1), 28-34.

91. Guo, H.-C.; Jin, Y.; Zhi, X.-Y.; Yan, D.; Sun, S.-Q., NLRP3 inflammasome activation by viroporins of animal viruses. *Viruses* **2015,** 7, (7), 3380-3391.

92. Nieto-Torres, J. L.; Verdiá-Báguena, C.; Jimenez-Guardeño, J. M.; Regla-Nava, J. A.; Castaño-Rodriguez, C.; Fernandez-Delgado, R.; Torres, J.; Aguilella, V. M.; Enjuanes, L., Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. *Virology* **2015,** 485, 330-339.

93. Wilson, L.; Mckinlay, C.; Gage, P.; Ewart, G., SARS coronavirus E protein forms cation-selective ion channels. *Virology* **2004,** 330, (1), 322-331.

94. Allen, I. C.; Scull, M. A.; Moore, C. B.; Holl, E. K.; McElvania-TeKippe, E.; Taxman, D. J.; Guthrie, E. H.; Pickles, R. J.; Ting, J. P.-Y., The NLRP3 inflammasome mediates *in vivo* innate immunity to influenza A virus through recognition of viral RNA. *Immunity* **2009,** 30, (4), 556-565.

95. Jo, E.-K.; Kim, J. K.; Shin, D.-M.; Sasakawa, C., Molecular mechanisms regulating NLRP3 inflammasome activation. *Cell. Mol. Immunol.* **2016,** 13, (2), 148-159.

96. Nieto-Torres, J. L.; DeDiego, M. L.; Verdiá-Báguena, C.; Jimenez-Guardeño, J. M.; Regla-Nava, J. A.; Fernandez-Delgado, R.; Castaño-Rodriguez, C.; Alcaraz, A.; Torres, J.; Aguilella, V. M., Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. *PLoS Pathog.* **2014,** 10, (5), e1004077.

97. Surya, W.; Li, Y.; Verdià-Bàguena, C.; Aguilella, V. M.; Torres, J., MERS coronavirus envelope protein has a single transmembrane domain that forms pentameric ion channels. *Virus Res.* **2015,** 201, 61-66.

98. Wilson, L.; Gage, P.; Ewart, G., Hexamethylene amiloride blocks E protein ion channels and inhibits coronavirus replication. *Virology* **2006,** 353, (2), 294-306.

99. Nieva, J. L.; Madan, V.; Carrasco, L., Viroporins: structure and biological functions. *Nature Reviews Microbiology* **2012,** 10, (8), 563-574.

100. Tosato, G.; Jones, K. D., Interleukin-1 induces interleukin-6 production in peripheral blood monocytes. *Blood* **1990,** 75, (6), 1305-10.

101. Cahill, C. M.; Rogers, J. T., Interleukin (IL) 1β induction of IL-6 is mediated by a novel phosphatidylinositol 3-kinase-dependent AKT/IκB kinase α pathway targeting activator protein-1. *J. Biol. Chem.* **2008,** 283, (38), 25900-25912.

102. Tisoncik, J. R.; Korth, M. J.; Simmons, C. P.; Farrar, J.; Martin, T. R.; Katze, M. G., Into the eye of the cytokine storm. *Microbiol. Mol. Biol. Rev.* **2012,** 76, (1), 16-32.

103. Zheng, H.; Fletcher, D.; Kozak, W.; Jiang, M.; Hofmann, K. J.; Corn, C. A.; Soszynski, D.; Grabiec, C.; Trumbauer, M. E.; Shaw, A., Resistance to fever induction and impaired acute-phase response in interleukin-1β-deficient mice. *Immunity* **1995,** 3, (1), 9-19.

104. Bode, J. G.; Albrecht, U.; Häussinger, D.; Heinrich, P. C.; Schaper, F., Hepatic acute phase proteins–regulation by IL-6-and IL-1-type cytokines involving STAT3 and its crosstalk with NF-κB-dependent signaling. *Eur. J. Cell Biol.* **2012,** 91, (6-7), 496-505.

105. Barrett, D. M.; Teachey, D. T.; Grupp, S. A., Toxicity management for patients receiving novel T-cell engaging therapies. *Curr. Opin. Pediatr.* **2014,** 26, (1), 43.

106. Grupp, S. A.; Kalos, M.; Barrett, D.; Aplenc, R.; Porter, D. L.; Rheingold, S. R.; Teachey, D. T.; Chew, A.; Hauck, B.; Wright, J. F., Chimeric antigen receptor–modified T cells for acute lymphoid leukemia. *N. Engl. J. Med.* **2013,** 368, (16), 1509-1518.

107. Xu, X.; Han, M.; Li, T.; Sun, W.; Wang, D.; Fu, B.; Zhou, Y.; Zheng, X.; Yang, Y.; Li, X., Effective treatment of severe COVID-19 patients with tocilizumab. *ChinaXiv* **2020,** 202003, (00026), v1.

108. Giamarellos-Bourboulis, E. J.; Netea, M. G.; Rovina, N.; Akinosoglou, K.; Antoniadou, A.; Antonakos, N.; Damoraki, G.; Gkavogianni, T.; Adami, M.-E.; Katsaounou, P., Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. *Cell Host & Microbe* **2020**.

109. Zhang, C.; Wu, Z.; Li, J.-W.; Zhao, H.; Wang, G.-Q., The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. *Int. J. Antimicrob. Agents* **2020**, 105954.

110. Dinarello, C. A., A clinical perspective of IL-1β as the gatekeeper of inflammation. *Eur. J. Immunol.* **2011,** 41, (5), 1203-1217.

111. Schlesinger, N., Anti-interleukin-1 therapy in the management of gout. *Curr. Rheumatol. Rep.* **2014,** 16, (2), 398.

112. Dinarello, C. A.; Simon, A.; Van Der Meer, J. W., Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nature reviews Drug discovery* **2012,** 11, (8), 633-652.

113. Maschalidi, S.; Sepulveda, F. E.; Garrigue, A.; Fischer, A.; de Saint Basile, G., Therapeutic effect of JAK1/2 blockade on the manifestations of hemophagocytic lymphohistiocytosis in mice. *Blood, The Journal of the American Society of Hematology* **2016,** 128, (1), 60-71.

114. DeDiego, M. L.; Álvarez, E.; Almazán, F.; Rejas, M. T.; Lamirande, E.; Roberts, A.; Shieh, W.-J.; Zaki, S. R.; Subbarao, K.; Enjuanes, L., A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated *in vitro* and *in vivo*. *J. Virol.* **2007,** 81, (4), 1701-1713.

115. Lamirande, E. W.; DeDiego, M. L.; Roberts, A.; Jackson, J. P.; Alvarez, E.; Sheahan, T.; Shieh, W.-J.; Zaki, S. R.; Baric, R.; Enjuanes, L., A live attenuated severe acute respiratory syndrome coronavirus is immunogenic and efficacious in golden Syrian hamsters. *J. Virol.* **2008,** 82, (15), 7721-7724.

116. Regla-Nava, J. A.; Nieto-Torres, J. L.; Jimenez-Guardeño, J. M.; Fernandez-Delgado, R.; Fett, C.; Castaño-Rodríguez, C.; Perlman, S.; Enjuanes, L.; DeDiego, M. L., SARS coronaviruses with mutations in E protein are attenuated and promising vaccine candidates. *J. Virol.* **2015**, JVI. 03566-14.

117. Fett, C.; DeDiego, M. L.; Regla-Nava, J. A.; Enjuanes, L.; Perlman, S., Complete protection against severe acute respiratory syndrome coronavirus-mediated lethal respiratory disease in aged mice by immunization with a mouse-adapted virus lacking E protein. *J. Virol.* **2013,** 87, (12), 6551-6559.

FIGURES and TABLES

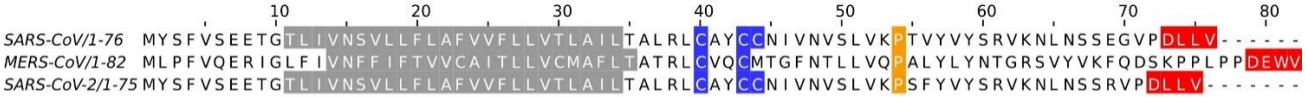

Figure 1: A sequence comparison of the envelope (E) protein amino acid sequences for the pathogenic human coronaviruses (hCoVs). The comparison was constructed using Jalview software (v 2.11.1.0). Important sequence features transmembrane domain (TMD) (gray), conserved cysteine (blue) and proline (orange) residues, and the PDZ-binding motif (PBM) (red) are indicated. The E protein reference sequences for SARS-CoV (Q19QW7), MERS-CoV (R9UQN1), and SARS-CoV-2 (YP_009724392.1) were obtained from the NCBI database.