



Endothelial Cells in Emerging Viral Infections

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There are several reasons to consider the role of endothelial cells in COVID-19 and other emerging viral infections. First, severe cases of COVID-19 show a common breakdown of central vascular functions. Second, SARS-CoV-2 replicates in endothelial cells. Third, prior deterioration of vascular function exacerbates disease, as the most common comorbidities of COVID-19 (obesity, hypertension, and diabetes) are all associated with endothelial dysfunction. Importantly, SARS-CoV-2's ability to infect endothelium is shared by many emerging viruses, including henipaviruses, hantavirus, and highly pathogenic avian influenza virus, all specifically targeting endothelial cells. The ability to infect endothelium appears to support generalised dissemination of infection and facilitate the access to certain tissues. The disturbed vascular function observed in severe COVID-19 is also a prominent feature of many other life-threatening viral diseases, underscoring the need to understand how viruses modulate endothelial function. We here review the role of vascular endothelial cells in emerging viral infections, starting with a summary of endothelial cells as key mediators and regulators of vascular and immune responses in health and infection. Next, we discuss endotheliotropism as a possible virulence factor and detail features that regulate viruses' ability to attach to and enter endothelial cells. We move on to review how endothelial cells detect invading viruses and respond to infection, with particular focus on pathways that may influence vascular function and the host immune system. Finally, we discuss how endothelial cell function can be dysregulated in viral disease, either by viral components or as bystander victims of overshooting or detrimental inflammatory and immune responses. Many aspects of how viruses interact with the endothelium remain poorly understood. Considering the diversity of such mechanisms among different emerging viruses allows us to highlight common features that may be of general validity and point out important challenges.

Keywords: endothelium, virus, SARS-CoV-2, inflammation, vascular dysfunction and damage, emerging infections

INTRODUCTION

As the human population expands, a closer proximity to wild animals and their habitat has resulted in a more frequent spillover of viral infections to humans and livestock. At the same time, our mobility has increased. This augments the risk that emerging infections can spread globally. As a result, evolving infections pose a significant threat to human and animal health and the global economy. This is clearly illustrated by the ongoing severe acute respiratory syndrome coronavirus

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2 (SARS-CoV-2) pandemic (>2.1 million deaths). Other recent epidemics include the 2014–2016 Ebola outbreak in West Africa (>11,000 deaths), the MERS-CoV outbreak in Saudi Arabia 2012–2019 (>850 deaths), and regular outbreaks of highly fatal Nipah virus infection in Bangladesh and surrounding countries, among others (1).

A characteristic feature of SARS-CoV-2 is that the virus, in addition to infecting airway epithelial cells, also infects endothelial cells (2–4). This infection pattern can be recapitulated in the laboratory, as SARS-CoV-2 infects and replicates in human capillary organoids (5). However, it is not known if replication in endothelial cells is a ubiquitous feature of disease or limited to certain patient groups or clinical presentations. Coronavirus disease 2019 (COVID-19) has an increased case fatality rate in individuals suffering from conditions associated with endothelial dysfunction (diabetes, hypertension, and cardiovascular disease) (6). Furthermore, typical clinical signs in severe COVID-19 are compatible with breakdown of vascular function (multi-organ endothelial damage, thrombosis and angiopathies, dysregulated inflammation, and pulmonary oedema) (2, 3, 7). To this end, it is highly relevant to address the role of the vascular compartment in COVID-19 (7, 8).

SARS-CoV-2's ability to infect endothelial cells is shared by many emerging viruses with great relevance to human and animal health. For example, henipavirus infection leads to widespread infection of endothelial and vascular smooth muscle cells, with extensive vasculitis and occasional endothelial syncytia formation (9, 10). In hantavirus pulmonary syndrome, there is severe dysregulation of vascular permeability and strong expression of viral antigens in the pulmonary microvasculature (11). Substantial endothelial expression of viral antigens is also observed in several emerging infections of animals, which have a major impact on animal welfare and sustainable food production. Highly pathogenic avian influenza virus (HPAIV, also relevant because of its zoonotic potential) (12) and pathogenic variants of infectious salmon anaemia virus (ISAV) (13) are prime examples of viruses that infect endothelial cells of production animals. Other viruses have a more generalised cell tropism, but show some degree of endothelial infection during natural infection. Relevant examples include the flaviviruses (such as dengue virus) (14) and filoviruses (Ebola and Marburg viruses) (15). **Table 1** provides a list of viral pathogens discussed in the current article and associated pathological findings of vascular disease. However, the complete list of all viruses that target the endothelium would be much more extensive.

In addition, severe viral disease more often than not includes signs related to dysregulated vascular function. Typical presentations include dysregulated blood flow, uncontrolled inflammation and vascular permeability, and microvascular thrombosis and bleeding. Importantly, vascular function may also be disturbed in the absence of endothelial infection. This can result from signalling from infected cells or collateral damage when immune cells attempt to limit infection. In conclusion, the question of how viruses target the endothelium, either directly or as bystanders, appears central to understand the pathogenesis of disseminated viral disease.

Complementary to several recent reviews that discuss the vascular component of COVID-19 [e.g., (8, 18, 19)], we here broaden our perspective to consider the diversity by which emerging viruses interact with endothelial cells. Briefly, we review features of endothelial cells relevant to their role in the pathogenesis of viral disease, summarise what is known about viral properties that regulate their ability to infect endothelial cells, and discuss how such tropism may affect the course of infection and disease development. Our aim is not to provide a complete overview of all viral interactions with endothelium, but rather to provide examples that illustrate the range of such interactions, highlight general principles, and point to unanswered questions related to disease development.

ENDOTHELIAL FUNCTION IN HOMEOSTASIS AND INFLAMMATION

Endothelial Cells Are Gatekeepers of the Blood-Tissue Barrier

Endothelial cells line the inner surface of all blood and lymphatic vessels. This single cell layer, the endothelium, creates a semi-permeable barrier between blood or lymph and its surrounding tissues. In an adult human, the vasculature contains $\sim 6 \times 10^{11}$ endothelial cells (20), covering a surface area of 4,000–7,000 m². The vascular network is a highly branched closed circulation that extends into all organs, nourishes every tissue, and provides a gateway for extravasation of fluids, solutes, macromolecules, hormones, and immune cells. Consequently, all possible entry ports of viral infection are in close contact with endothelial cells. A good example is the lower respiratory tract, where intimate apposition between alveolar epithelial and lung microvascular endothelial cells is required for efficient alveolar gas exchange. Microvascular beds, composed of arterioles, capillaries, and post-capillary venules, make up the greatest surface of the vascular circulation by far and is where most physiological processes brought on by the endothelium occur (21). This is also where most virus-induced vascular pathology manifests.

Endothelial Profiles Vary Between Vascular Beds, Branches, and Activation States

Endothelial cells show tissue- and vascular bed-specific profiles that are likely to regulate their susceptibility and permissibility to viral infection (22). For example, the observation of dengue antigens in liver sinusoids (14) during natural infection should be considered in light of the dengue virus' ability to exploit scavenger receptors for internalisation (23, 24). The vast diversity among endothelial cells arises through a combination of inputs that impose phenotypes of variable durability (25). Some phenotypes are mitotically stable, like the epigenetic modifications that define arterial and venous cells (26, 27). Others provide memories of past stimulations, like biologically active proteins with long half-lives stored in Weibel-Palade bodies (28). Finally, endothelial phenotypes may also be highly dynamic, like tip-stalk cell phenotypes in angiogenic sprouting (29). Similar to many aspects of endothelial heterogeneity, the ability to regulate critical vascular functions is dynamic and arises from cues within

TABLE 1 | Viruses that infect endothelial cells and related vascular pathological findings.

Virus (Family)	EC infection	Vascular pathological features	References
SARS-CoV-2 (coronaviridae)	One of several cell types infected	Intra-endothelial virus particles in all examined tissues; Multi-organ vascular involvement; Endotheliitis with perivascular T cell and mononuclear cell infiltrates; Severe endothelial injury with disrupted cell membranes, and apoptotic bodies; Widespread thrombosis, congestion, and microangiopathy; Dysregulated inflammation	(2–4)
Nipah and Hendra henipavirus (paramyxoviridae)	Major cell type infected	Widespread expression of viral antigens in endothelial and vascular smooth muscle cells; Multi-organ vascular involvement; Endotheliitis affecting small arteries, arterioles, capillaries, and venules; Focal neutrophil and mononuclear infiltrates; Severe endothelial injury with segmented destruction, mural necrosis, and fragmented nuclei; Necrosis and haemorrhages adjacent to damaged endothelium; Endothelial syncytia, multinucleated giant cells	(9, 10)
Hantavirus (hantaviridae)	Major cell type infected	Viral antigens, viral inclusions bodies, and virus particles in pulmonary capillary and small vessel endothelium; Less frequent presence of endothelial viral antigens in extra-pulmonary tissues, with kidney being the most affected location; Pulmonary and generalised vascular congestion, but histopathological changes confined to lungs; Alveolar and interstitial oedema; Mononuclear cell infiltrates without visible endotheliitis; Occasional swollen or enlarged endothelial cells, but no evidence of necrosis or microvascular thrombosis	(11)
Influenza A, H5N1, H7N1 (orthomyxoviridae)	Major cell type infected	Viral antigens in blood and lymphatic endothelial cells of all examined tissues; Viral particles budding from the luminal side; Microvascular thrombosis; Multi-organ vascular involvement with widespread necrotic and haemorrhagic foci and oedema; Inflammation, except in per-acute disease	
Infectious salmon anaemia virus (orthomyxoviridae)	Major cell type infected	Viral antigens and virus particles in endothelial cells of the primary and secondary vascular system; Multi-organ vascular involvement, including bleeding, congestion, and oedema; Severe anaemia; Absence of perivascular leukocyte infiltrates	
Ebolavirus (filoviridae)	One of many cell types infected	Endothelial infection becomes evident after onset of symptoms; Intra-endothelial viral inclusion bodies and virus particles; Absence of inflammation and morphological evidence of EC damage; Petechiae and ecchymoses on skin and mucous membranes, internal haemorrhages, fibrin deposition suggestive of consumptive coagulopathy; Multi-organ necrotic foci	(15, 16)
Dengue virus (flaviviridae)	One of many cell types infected	Viral antigens in endothelial cells of lung, liver, and heart; Diffuse haemorrhage, oedema, and focal necrosis; Mononuclear cell infiltrates	(14, 17)
Zikavirus, West Nile virus (flaviviridae)	Limited evidence of <i>in vivo</i> endothelial infection		

the extracellular environment (28, 30). Thus, maintaining blood fluidity, regulating blood flow, controlling trans-endothelial extravasation of plasma proteins, and controlling leukocyte trafficking all respond to signals generated during homeostasis, hypoxia, inflammation, and repair. Importantly, endothelial cells may reside in a resting phenotype over long periods of time and still maintain the ability to orchestrate dramatic responses when activated (31).

Endothelial Cells Are Central Regulators of Vascular Function

Endothelial cells control vascular functions, and tissue damage or infection stimulates an endothelial phenotype that supports the protective host response. However, overwhelming or persistent activation can lead to endothelial dysfunction and contribute to pathology (Figure 1).

Regulation of Blood Flow

Arterial endothelium plays an essential role in regulating blood flow by controlling perivascular smooth muscle cell contraction. The specific blood-flow mediators released by endothelial cells vary in time and space, but the principal vaso-relaxant is

nitric oxide (NO) (32). NO is also linked to various other endothelial functions, and reduced NO bioavailability is a central feature of endothelial dysfunction (33). Indeed, a non-invasive test for measuring the NO-mediated response to alterations in blood pressure (brachial artery flow-mediated dilation, FMD) is commonly used to define endothelial dysfunction (34). In a recent position paper by the European Society for Cardiology, FMD was proposed as a potential tool for risk stratification in COVID-19 (7). The renin-angiotensin system is also a key player in the regulation of blood pressure and organ perfusion. Excessive activation of the renin-angiotensin system by angiotensin II (ANG-II) causes vasoconstriction, as well as vascular and cardiac damage. However, its counterregulation by the angiotensin converting enzyme 2 (ACE2), the surface protein used by SARS-CoV-2 for cellular attachment (35–37), and its products, angiotensin 1-7 and angiotensin 1-9, balances signalling to promote the perfusion of central organs like heart and kidney (38).

Maintenance of Blood Fluidity

Under homeostatic conditions, the endothelium actively inhibits coagulation (32). Because microvascular thrombosis is a clinical

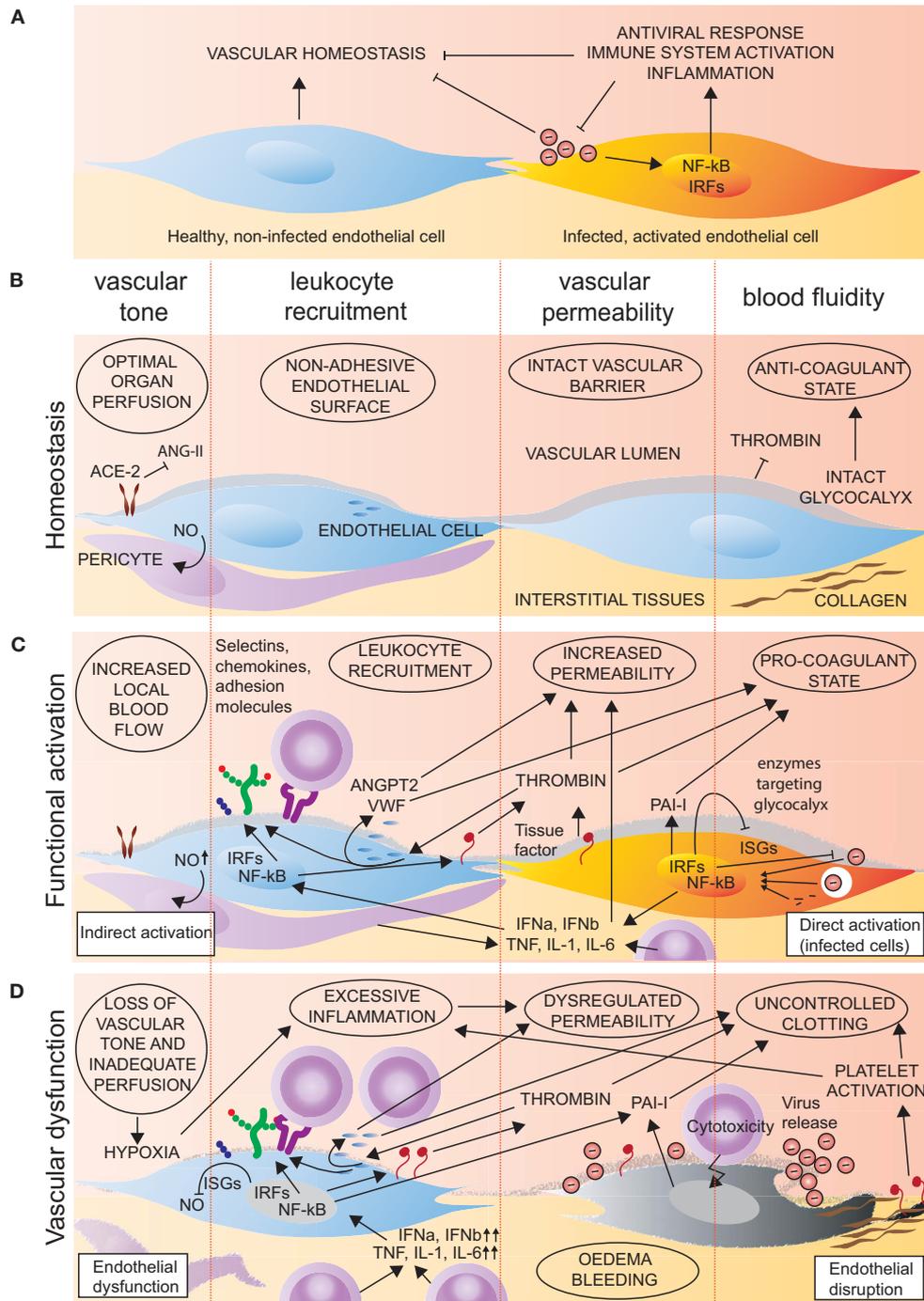


FIGURE 1 | Endothelial function in homeostasis and viral infection. **(A,B)** In homeostasis, endothelial cells promote a non-adherent vascular surface that promotes tissue perfusion, limits inflammation and the transvascular movement of cells and proteins, and prevents clotting. **(A,C)** Upon viral infection or tissue damage, endothelial cells are activated directly by viral components or indirectly by soluble mediators (IL-1, TNF, IL-6, IFN α , IFN β). Such activation initiates signalling that culminates in activation and nuclear translocation of IRF and NF- κ B transcription factors. This results in expression of interferon-stimulated genes (ISGs) and inflammatory mediators (e.g., tissue factor, selectins, chemokines, and adhesion molecules) that promote antiviral responses, clotting, vascular permeability, and leukocyte recruitment. In addition, type I activation of endothelial cells, for example by thrombin, causes a rapid release of pre-synthesised molecules (e.g., VWF, ANGPT2, P-selectin, and CXCL8) with similar effects. **(A,D)** In the case of overwhelming or persistent activation, endothelial cells may become dysfunctional, resulting in loss of control of vascular tone, permeability, and coagulation. In addition, direct viral damage to the endothelium further disrupts the vascular barrier and exposes pro-thrombotic factors (tissue factor, collagen) that exacerbate dysfunctional coagulation.

feature of many viral infections and central to COVID-19 (39), we here provide a brief overview of the mechanisms involved. First, endothelial cells bind and present tissue factor pathway inhibitors that block the action of the factor-VIIa-tissue-factor complex (32). Second, endothelial cells produce and display heparan sulphate proteoglycans in the glycocalyx that bind anti-thrombin III, enabling inhibition of thrombin molecules generated by the coagulation cascade (32). Third, endothelial cells synthesise and present thrombomodulin, a cell surface-bound protein that prevents thrombin from cleaving fibrinogen, a key step in blood clot formation. Furthermore, this thrombomodulin-bound thrombin efficiently activates protein C, generating an enzyme that destroys certain clotting factors, also inhibiting coagulation (32). Fourth, thrombin inactivation by endothelial cells prevents platelet activation, another critical element in maintaining blood fluidity. Endothelial cells also produce and sequester von Willebrand factor (VWF) in Weibel-palade bodies. VWF is a large multimeric glycoprotein that plays a central role in coagulation, among others by strengthening the binding between platelets and collagen in the basement membrane (32). Finally, endothelial-produced NO, prostacyclin, and prostaglandin E2 synergistically inhibit platelet adhesion and aggregation (32). Importantly, in response to pro-inflammatory activation, endothelial cells change from an anti-coagulant to a pro-coagulant phenotype (**Figure 1C**). Increased tissue factor expression and exposure of extracellular matrix components like collagen initiate the extrinsic coagulation cascade activation and increased production of thrombin and fibrin. The resulting pro-thrombotic state is further exaggerated by an elevation of plasminogen activator inhibitor 1 (PAI-1) that suppresses the fibrinolytic system (40). Finally, secretion of VWF promotes platelet activation and additional propagation of the coagulation cascade.

Control of Vascular Permeability

Microvascular endothelial cells also regulate the extravasation of fluids, solutes, and plasma proteins. Accordingly, dysregulation of microvascular function mediates oedema and bleeding in viral disease (41). The maintenance of microvascular integrity relies on crosstalk between two key components of the endothelium, the glycocalyx and the intercellular junctions. First, the luminal surface of all vascular endothelial cells is covered by the glycocalyx. This is a thick hydrated layer of glycoproteins with acidic oligosaccharides and terminal sialic acids that confer a net negative charge (42). Of note, these highly abundant sialic acids are frequently exploited by viruses to attach to the cellular surface (43). The glycocalyx also contains membrane-bound proteoglycans with associated glycosaminoglycans like heparan sulphates (42). It forms a gel-like interface between the plasma membrane and the blood that is central to maintaining proper barrier function, and its disruption leads to vascular leakage (42, 44). Second, the conformation of endothelial intercellular junctions is highly dynamic and responds to a range of stimuli (45). Tight junction molecules regulate paracellular permeability to ions and small molecules, and are therefore most prominent at sites where strict regulation of vascular permeability is essential, like the blood-brain barrier (46). In contrast, adherens

junction molecules are evenly distributed along the vascular tree, and mediate permeability to large molecular weight plasma components (46). Adherens junctions are also hubs for the integration of cell-cell adhesion, cytoskeletal reorganisation, and intracellular signalling (45). The highly endothelial cell-specific vascular endothelial (VE)-cadherin (47) is a central component of adherens junctions and has a crucial role in regulating the permeability to plasma proteins across intact endothelium (48).

Orchestration of Leukocyte Recruitment

Venular endothelial cells keep tight control of leukocyte trafficking from blood to tissues and orchestrate local inflammatory responses, for example in viral pneumonia. For leukocytes to cross the vessel wall, they must first adhere to the endothelial surface. This process takes place in post-capillary venules of inflamed tissues and has been entitled the multistep adhesion cascade (49). Briefly, the capture and rolling of leukocytes on endothelial surfaces is mediated by P-, E-, and L-selectins, which bind glycosylated ligands. This happens in a dynamic process, forming and breaking bonds that support capture and rolling under shear stress (50). P- and E-selectin appear to be the most important selectins expressed on activated post-capillary venules in non-lymphoid tissues (51). Rolling leukocytes gets into close contact with endothelial cells, and this proximity allows inside-out activation of leukocyte integrins by glycocalyx-presented chemokines. In addition to producing chemokines themselves, endothelial cells are also able to capture chemokines produced by stromal cells, transcytose them, and present them on their luminal surface (52). This transcytosis of chemokines communicates the nature of extravascular inflammatory processes to circulating leukocytes and is mediated by atypical chemokine receptors. A key example is the Duffy antigen receptor for chemokines that binds both CC and CXC inflammatory chemokines and is required for optimal chemokine-induced leukocyte transmigration (52). Cytokines produced by resident leukocytes also stimulate endothelium to sequentially upregulate adhesion molecules. These adhesion molecules bind chemokine-activated leukocyte integrins and mediate firm adhesion and arrest.

Endothelial Activation May Be Rapid or Sustained

Endothelial responses to inflammatory stimuli can be classified as type I (in response to e.g., thrombin) or type II (in response to inflammatory cytokines). Type I responses rely on the release of pre-synthesised molecules and do not depend on new gene expression. This very rapid activation allows recruitment of leukocytes within minutes of the initiating stimulus (53). Type I responses are typically mediated by G protein-coupled receptors, whose transient signalling ensures the short-lived nature of the response (32). G-protein coupled signalling results in elevation of cytosolic free Ca^{2+} . Free Ca^{2+} interacts with calmodulin to drive the phosphorylation of myosin light chain that initiates the exocytosis of the predominant endothelial storage granule, the Weibel-Palade body. In addition to the pro-thrombotic molecule VWF, a subset of Weibel-Palade bodies also contains angiopoietin 2 (ANGPT2) (54), P-selectin (55), and the powerful neutrophil chemoattractant CXCL8 (56) that together drive

vascular destabilisation and rapid leukocyte recruitment. The rise in intracellular Ca^{2+} also leads to NO and prostacyclin production, thus promoting increased blood flow and vascular leakage. In contrast, type II responses constitute the classical response to pro-inflammatory cytokines, like interleukin (IL)-1 and tumour necrosis factor (TNF), resulting in activation of the inflammatory transcription factors NF- κ B and AP1 and *de novo* gene expression. Such activation gives rise to a more sustained and efficient inflammatory response, but with the same consequences of increased blood flow, vascular leakage, and a coordinated leukocyte recruitment (**Figure 1C**). Moreover, both type I and type II activation of endothelial cells increase vascular permeability, at least in part by stimulating the formation of fibrillar adhesions to the extracellular matrix that destabilise endothelial adherens junctions and allow extravasation of plasma proteins (57).

Endothelial Signalling Pathways That Regulate Normal Vascular Function

In addition to signalling induced by inflammatory cytokines, a number of signalling pathways integrate to regulate endothelial function, including angiopoietin-1 (ANGPT1)/TIE2- (58), vascular endothelial growth factor (VEGF)- (59), WNT/ β -catenin-, sphingosine-1-phosphate (S1P)-, and platelet-derived growth factor (PDGF)-signalling (60). In homeostasis, such highly interlinked signalling promotes stable endothelial junctions, as well as interactions with neighbouring mural cells. Endothelial junctional stability primarily relies on VE-cadherin homophilic interactions (45, 46). In a resting vessel, VE-cadherin also directly interacts with VEGFR2, reducing its signalling potential and protecting the cell from the destabilising effect of VEGF-A (61). Interestingly, in this setting, VEGF-A mediates anti-apoptotic signalling through PI3K/AKT-induced eNOS phosphorylation; exemplifying the complexity of the signalling involved in endothelial cell maintenance (62). VEGFR2 and VE-cadherin also merge in a mechano-sensory complex together with platelet EC adhesion molecule 1 (also known as CD31) that mediates the endothelial response to shear stress (63). Moreover, ANGPT1/TIE2-, S1P-, and PDGF-signalling enhance pericyte recruitment and integrin-mediated adhesion to the extracellular matrix, further stabilising vessel integrity (64–67). Finally, endothelial cells also express connexins that form intercellular gap junctions and contribute to the close communication between adjacent endothelial cells, pericytes, and vascular smooth muscle cells (68, 69).

Endothelial Dysfunction Is a Central Component of Many Viral Syndromes

A common feature of viruses that infect endothelial cells is their ability to cause severe multi-organ disease. The clinical features of end-stage viral disease are often similar, with hypoperfusion, oedema, bleeding, and thrombosis, all indicating breakdown of central vascular functions (**Figure 1D**). Typical vascular-associated histopathological features of different viral infections are summarised in **Table 1**. This section reviews common viral syndromes, with emphasis on their vascular component.

Acute Respiratory Distress Syndrome

In all virus pandemics of the last two decades (Influenza A H1N1; SARS-CoV, MERS-CoV, and SARS-CoV-2) a high proportion of affected patients have developed severe illness and acute respiratory distress syndrome (ARDS) (70–72). ARDS is a complication to viral pneumonia, characterised by pulmonary oedema, hypoxaemia, and increasing respiratory failure, resulting in progressive multi-organ failure and often fatal outcomes [exceeding 50% (73)]. Breakdown of the pulmonary microvascular barrier function is a central feature of ARDS (74, 75). The histopathological correlates are disruption of the alveolar epithelial and vascular barriers and accumulation of protein-rich exudate within the interstitium and alveolus (76). This eventually leads to diffuse alveolar damage (77, 78), which is the pathologic hallmark of ARDS. Respiratory viruses initially infect the upper airway epithelium, but the importance of this step to alveolar damage is debated. Instead, it might be that the epithelium merely functions as a portal of entry, while the alveolar damage primarily results from compromised vascular integrity (79). The release of potent pro-inflammatory cytokines and chemokines by resident macrophages, epithelial, and endothelial cells leads to the recruitment of both innate and adaptive immune cells (80). These cells cause further exacerbation of tissue damage. Specific cytokine profiles vary by the virus, but a general phenomenon for all recent pandemic viruses is their ability to induce an excessive early cytokine response (81, 82). Together with immune cell recruitment, this excessive response strongly correlates with poor outcome. In H1N1 infection, pulmonary endothelial cells play a central role in regulating both innate immune cell recruitment as well as innate cytokine and chemokine production (83). Hence, it is tempting to speculate if this also holds true for coronavirus-induced ARDS. A study comparing post mortem-collected tissues from H1N1 and COVID-19 patients found more severe disruption of endothelial morphology in COVID-19 patients (3), supporting this hypothesis. Clinical features of the hantavirus pulmonary syndrome very much mimic those of influenza- and coronavirus induced ARDS (11). Nevertheless, it does not appear to be mediated by release of inflammatory cytokines or morphological disruption of the endothelium (11, 41). Instead, the dramatic disruption of vascular barrier function is mediated by direct viral manipulation of inter-cellular junctions (41, 84), discussed in more detail in section 4.3.1.

Microvascular Thrombosis and Endotheliitis

Disseminated intravascular coagulation and thrombotic microvascular occlusion are complications of severe systemic inflammation and a sequela to most infections discussed in this article (**Table 1**). Notably, COVID-19 has been strongly associated with thrombosis (85), more so than other coronaviruses and H1N1 (39). This is not entirely surprising, as severely ill SARS-CoV-2-infected patients have intra-endothelial viral inclusion bodies, endothelial apoptosis, and inflammation (endotheliitis) (2–4). The extensive morphological vascular changes affect a range of organs, from the lung to the gastrointestinal tract, suggesting widespread endothelial activation and damage. The true prevalence of microthrombosis

in COVID-19 is still unknown, as most studies to date do not include systematic and comprehensive investigation protocols (39). However, multiple reports have shown cumulative incidences of thrombotic events around 50% (86), even when thrombo-prophylaxis has been administered. In fact, in one study 56% of patients receiving full anticoagulation were diagnosed with pulmonary embolism (87). Interestingly, these rates are similar to those reported in patients with severe sepsis and shock (39, 88).

Systemic Inflammatory Response Syndrome and Multi-Organ Failure

As the disruption of vascular functions becomes more severe, many viral infections culminate in a typical systemic inflammatory response syndrome, resembling severe sepsis. In COVID-19, it soon became evident that patients with severe disease fulfilled all diagnostic criteria for sepsis and septic shock, even in the absence of bacterial co-infections (89). This systemic inflammatory response syndrome reflects overwhelming activation by inflammatory cytokines and other activators. Initial peripheral vasoconstriction is followed by uncontrolled vascular dilation, functional hypovolaemia, and circulatory failure, eventually culminating in multi-organ failure and death. Nevertheless, cytokine levels in critically ill COVID-19 patients were lower than in patients with bacterial sepsis (90). Therefore, it is possible that the severe direct viral damage to the endothelium (3) further adds to the cytokine-stimulated breakdown of vascular function in COVID-19. This parallel between bacterial and viral sepsis is maintained in dengue vascular permeability syndrome. This syndrome is characterised by severe hypovolemic shock, in addition to disruption of haemostasis. Damaged endothelial cells are unable to contain macromolecules and fluid in the circulation, and there is a dramatic reduction in blood volume and a corresponding increase in haematocrit (91). Nevertheless, after a critical period of 24–36 h, vascular function is restored and a rapid return to homeostasis ensues (91). This vascular leak appears to rely mainly on endothelial activation by the viral NS1 protein (92) via TLR4 (92). Interestingly, TLR4 is also the receptor for LPS, a bacterial endotoxin and a key driver of sepsis (93).

Kawasaki Disease

While children appear to be relatively resistant to the respiratory complications of COVID-19, SARS-CoV-2 has been linked with a recent rise in the occurrence of Kawasaki disease (94). Kawasaki disease is a rare multi-system inflammatory response syndrome and vasculitis. It occurs in young children and is believed to arise due to post-viral immune reactions. SARS-CoV-2-mediated Kawasaki disease appears to have several distinct features, including severe haemodynamic instability and associated myocarditis (95). Localised dermal vasculitis associated with intra-endothelial SARS-CoV-2 particles has also been reported in children (4). Interestingly, these children tested negative for the virus by standard qPCR, but retained SARS-CoV-2-like particles inside endothelial cells in lesional areas that also stained positive for SARS-CoV-2 spike protein (4).

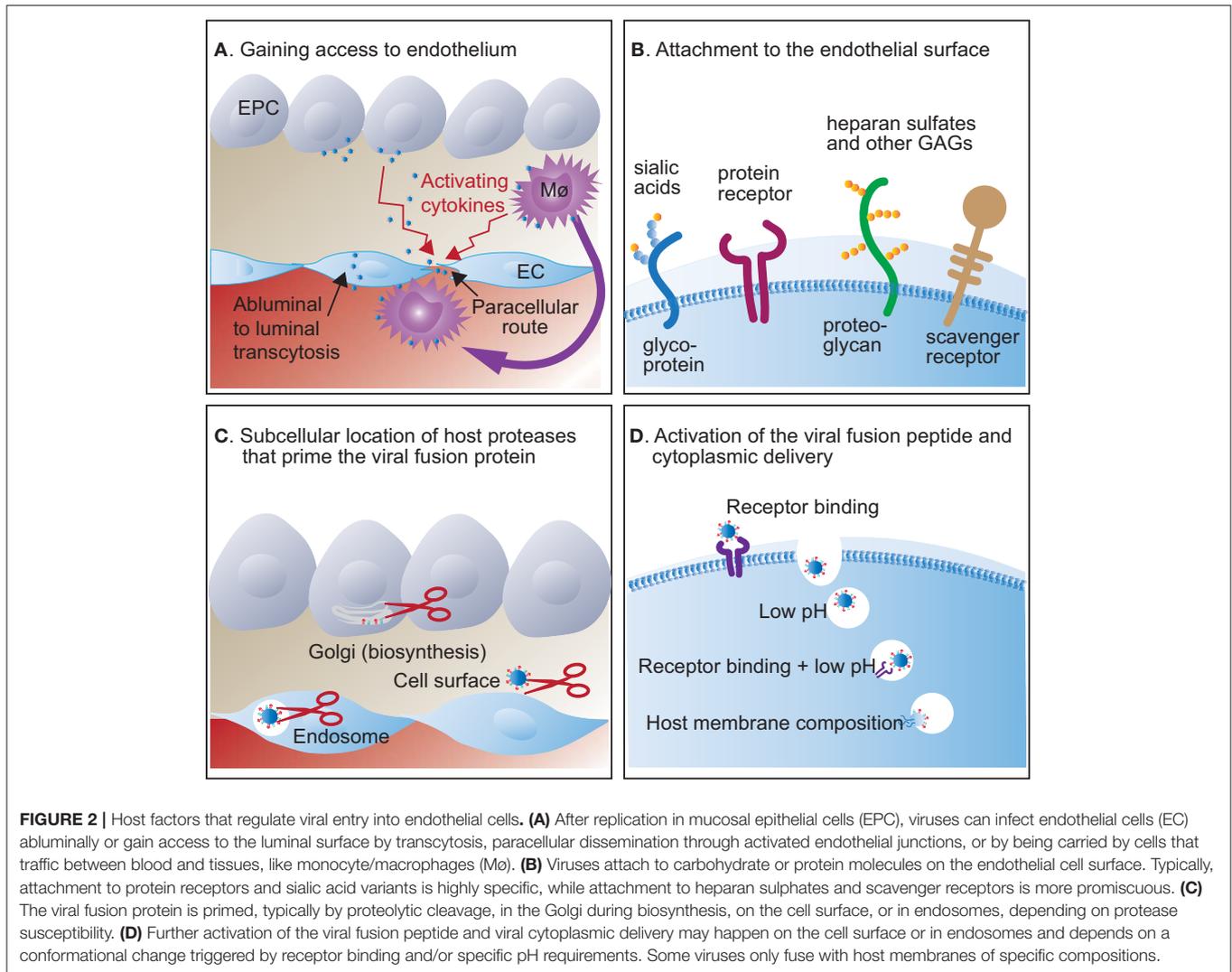
ENDOTHELIOtropISM MAY INFLUENCE THE OUTCOME OF VIRAL INFECTION

Endotheliotropism

The tropism of a pathogen is the host, cell type or tissue that supports its growth and, in the context of a virus, the generation of new progeny. Hence, an endotheliotropic virus is defined by its ability to attach to, enter, and replicate in endothelial cells. Such tropism depends on several factors (summarised in **Figure 2**) that will be discussed in detail in section 2.10. For viruses like SARS-CoV-2 (2), henipavirus (9), hantavirus (11), HPAIV (12), and ISAV (13), examination of tissues from infected individuals provides strong evidence of endotheliotropism (**Table 1**). The observation of intracellular virus particles by electron microscopy, endothelial expression of viral proteins by immunostaining, and/or endothelial viral RNA production by *in situ* hybridisation shows that endothelial cells are targeted during infection. Importantly, the specificity of reagents detecting viral antigens and nucleotides can be ensured by including tissues from non-infected individuals. Single-cell RNA-sequencing and analysis of enriched cell populations from digested tissues can also be used to map the presence of virus in individual cell types, again with a high specificity. However, sensitivity is probably a limiting factor of all these techniques, as cells with a low viral load may evade detection. Furthermore, neither of the techniques discussed above provide definitive evidence of generation of infective viral particles, although the observation of budding viral particles by electron microscopy is a strong indication. To evaluate whether infective progeny is produced requires serial passaging of virus, most often in cell culture. Conversely, the ability to replicate in cultured endothelial cells does not prove that the virus is endotheliotropic during natural infection. For example, while both Zikavirus and West Nile virus readily replicates in cultured endothelial cells (96, 97), endothelial expression of viral antigens does not appear to be a prominent feature of human infection.

Challenges Associated With Studying Endothelial Viral Infections

The discrepancy between observations of replication in cell culture and the lack of endothelial expression of viral antigens during natural Zikavirus and West Nile virus infections, underscores a highly relevant challenge. Often, detailed insight in the molecular processes of viral infection requires work in cellular model systems. Because many phenotypic traits of endothelial cells rely on input from their surroundings, endothelial cells drift when they are removed from their natural microenvironment (25). Hence, cultured endothelial cells may show a different repertoire of surface proteins and other modulators of viral infection than endothelial cells in tissues, leading to different outcomes. Moreover, culture systems do not reflect the complex interplay between stromal and immune cells that makes up the host defence to infection. Hence, the design and interpretation of cell culture experiments should always be done with a view to patient material analyses. However, working with patient samples also imposes challenges. For highly pathogenic and contagious agents, the proportion of deceased patients that undergo a full



autopsy may be low and could lead to bias. Moreover, analyses of unfixed tissues must be performed under high-level biosecurity restrictions, imposing substantially increased costs and limited availability of specialised equipment. Another challenge is that endothelial cells reside in tissues. Hence, sampling of material from human infections commonly originates from the terminal stage of the disease and may be poorly suited to study early events or even the peak viremic phase of infection. However, endothelial function can be evaluated non-invasively in patients by FMD (see section 2.3.1), making it possible to study viral effects on endothelial function at all stages of infection. Experimental infection of laboratory animals is often used to help our understanding of infection and disease development, including the sequential infection of specific cell populations. Because the pathogenesis of disease often differs between species, animal models of human viral infections have important limitations. Hence, the study of animal viruses in their natural hosts may have an important complementary role in studying host-pathogen interactions. In conclusion, several approaches should be used

to determine the target cell-type repertoire of a virus. Moreover, while the use of model systems is essential to obtain information at high resolution, results should always be interpreted in the context of observations from natural infections.

The Ability to Infect Endothelial Cells Is Associated With Virulence

For some viruses, the conversion from a well-tolerated to a virulent phenotype involves modulations of viral surface proteins that make the virus able to infect endothelial cells. Well-studied examples include avian influenza viruses of the H5 and H7 subtypes and ISAV, all belonging to the orthomyxovirus family. While low-pathogenic avian influenza virus (LPAIV) only replicates in mucosal epithelial cells, the conversion of H5 and H7 strains that defines HPAIV is associated with a switch in cellular tropism (12, 98). Typically, infection of susceptible bird species (domestic poultry and black swans) with HPAIV results in a global vascular infection pattern (12, 99, 100), where immunostaining of viral antigens gives a stronger

signal in endothelial cells than in epithelial cells of the same organ (100). Accordingly, HPAIV strains efficiently replicate in cultured human endothelial cells (101). One should note that viral replication in endothelial cells does not appear to be a prominent feature of influenza virus infection in humans and other mammals (12, 102). Nevertheless, the introduction of a miR-126 binding site that specifically prevented viral replication in endothelial cells, restricted H5N1 replication to the lung in experimentally infected mice and ameliorated disease in both mice and ferrets (103). In contrast, preventing viral replication in haematopoietic cells did not affect disease development (103). While only coming out of a single study, these findings suggest that the ability to infect endothelium may play a role in the extra-pulmonary dissemination and development of generalised H5N1 infection, also in mammals. Interestingly, single-cell RNA-sequencing of mouse lung after infection with the pandemic 2009 H1N1 influenza virus revealed viral mRNA transcription in 40–50% of endothelial cells (104). Nevertheless, the level of transcription in individual endothelial cells was low, raising the question of whether it would be detected by conventional methods like immunohistochemistry and *in situ* hybridisation. Like avian influenza virus, ISAV exists in two variants. Similar to LPAIV, the ubiquitous non-pathogenic HPR0 variant only infects epithelial cells at mucosal surfaces and cannot invade the interior of the fish (105). However, specific modulations of surface glycoproteins induce a dramatic increase in ISAV pathogenicity accompanied by a switch in cellular tropism and multi-organ infection of vascular endothelial cells (13). Together, these observations suggest that the ability to infect endothelium may be a critical factor in supporting generalised viral dissemination and promote disease.

The routes by which viruses reach endothelial cells during natural infection (**Figure 2A**) have not been completely mapped. One interesting question is the extent to which endothelial cells are infected from the abluminal tissue surface (**Figure 2A**). Considering a model where endothelial infection facilitates viral spread from mucosal sites to the circulation, viral endothelial entry would be expected to be abluminal. While some virus receptors, like integrins (106), are abundantly expressed on both abluminal and luminal endothelial surfaces, both influenza virus (107–109) and ISAV (13, 110) attach to cells by binding sialic acids that are mainly expressed in the glycocalyx on the luminal surface of endothelial cells (42). Furthermore, work in polarised endothelial cultures shows that HPAIV infects cells most efficiently from the luminal side (111). However, to infect endothelium from the luminal cell surface, the virus must cross the vascular barrier by other means. These could include abluminal-luminal endothelial transcytosis or diffusion across intercellular junctions, perhaps promoted by inflammation or injury that increases vascular permeability. Moreover, trafficking cells may carry virus between peripheral tissues and the circulation [discussed in (112)]. An excellent example of the latter is Nipah virus that attaches to lymphocytes that do not support its replication, but act as carriers and promote endothelial trans-infection (113). Interestingly, budding of H5N1 and ISAV mainly occurs from the luminal endothelial surface (13, 99, 114), suggesting that endothelial-produced viral particles, at least for

these viruses, predominantly enter the circulation rather than being released to surrounding tissues (115).

It should be noted that many viruses cause devastating pathology at the site of initial infection even in the absence of spread to distant anatomical sites, exemplified by the extensive pulmonary damage caused by SARS-CoV-2 and seasonal human influenza. Hence, endotheliotropism is by no means the only or even the major determinant of virulence.

The Ability to Cross Specific Vascular Beds Contributes to Viral Tissue-Tropism

Infection of endothelial cells may also allow circulating virus particles to enter tissues, provided that the virus can bud from or be transcytosed to the abluminal endothelial surface. Again, alternative routes exist to enter tissues from the blood stream, including diffusion through activated or compromised endothelium, endothelial transcytosis, or a Trojan-horse approach relying on infection of cells that traffic between blood and tissues (112). The relative importance of these pathways remains unclear.

Arthropod-borne viruses of the flavivirus family, like Zikavirus and West Nile virus, transmit by mosquito or tick bites and gain more or less direct access to the circulation. These viruses provide interesting examples of how circulating viruses target specific organs and give rise to distinct patterns of disease. Both viruses primarily cause pathology in the central nervous system, thus, the ability to cross the blood-brain barrier is central to pathogenesis. In addition, the ability to cross the placenta is highly relevant to Zikavirus pathology, where severe viral damage is restricted to the foetus. In the early phase of infection, the affinity for endothelial surface receptors appears to be a key factor in determining these agents' tissue-tropism (96, 116, 117). So far, there is limited evidence that Zikavirus and West Nile virus replicate in endothelial cells during natural infection. Their ability to cross intact endothelium probably relies on properties that allow attachment and transcytosis across the intact endothelial barrier, rather than productive infection (116, 117). Moreover, when the infection progresses, and inflammatory responses accelerate, increased vascular permeability appears to promote viral egress further, exemplified by the peripheral TLR3-dependent inflammation that facilitates West Nile virus entry into the brain (118). Also, several flaviviruses, including Zikavirus and West Nile virus, selectively disrupt the endothelial glycocalyx of their target tissues via secreted viral proteins, resulting in increased tissue-specific vascular permeability (119) that may also facilitate the entry of circulating viral particles.

Could Endothelial Infection Augment Viral Amplification?

The importance of viral replication in endothelial cells remains less clear. Nevertheless, the amelioration of disease in mouse and ferret models of H5N1 influenza upon endothelial-specific inhibition of viral replication (103) suggests that replication in endothelial cells at least in some cases promotes a more severe disease. Considering the abundance of endothelial cells in the human body, estimated to make up ~20% of the total number

of nucleated cells (20), we propose that viral amplification in endothelial cells deserves further study.

Altogether, there is substantial evidence that the ability to attach to, infect and/or travel across endothelial cells by transcytosis contributes to dissemination of viral infection and disease severity (42).

SPECIFIC FACTORS THAT DETERMINE THE ABILITY TO INFECT ENDOTHELIAL CELLS

Viral Attachment to Endothelial Cells Ranges From High to Low Specificity

The first step of the infectious cycle is the attachment of virus particles to the host target cell membrane. This process is mediated by interactions between viral surface glycoproteins and their corresponding cellular receptors (107, 120, 121). The endothelial glycocalyx presents a range of molecules involved in viral attachment and entry (Figure 2B). The binding of viral glycoproteins to such glycan and protein receptors is often highly specific and contributes to determining cellular and host specificity (122), although the presence of a viral receptor is not sufficient to allow infection. Patterns of virus binding can be mapped directly by virus binding assays [e.g., (13, 123)] or indirectly by mapping the expression of specific viral receptors by immunostaining. Lately, large datasets from single-cell RNA-sequencing experiments have been used to map the expression of receptors and proteins involved in SARS-CoV-2 attachment and entry by different cellular subsets (124, 125). Nevertheless, information from such analyses must be considered with the reservation that the correlation between mRNA and protein expression depends on several factors, including posttranslational modifications and protein half-life (125, 126). It should also be kept in mind that even when expressed at low levels, receptors may still be present in sufficient amounts to support viral entry. Hence, the expression level of a viral receptor should be regarded in the context of its binding affinity for the virus attachment glycoprotein. While low-affinity binding, as observed between influenza virus hemagglutinin (HA) and sialic acids, requires the abundant presence of the receptor for a high avidity association, a virus that binds its receptor with high affinity may require much lower expression levels of the receptor to support robust attachment (120).

Coronaviruses attach to host cells by their surface-exposed spike (S) glycoprotein that forms the characteristic crown shape that gave name to this virus family (127). The S protein consists of two non-covalently attached subunits, the S1 and S2, responsible for attachment and fusion, respectively (19). The N-terminal and C-terminal domains of the coronavirus S1-protein bind carbohydrate and protein receptors, respectively, and coronaviruses vary widely in their choice of attachment receptors, including both *O*-acetylated and non-acetylated sialic acids and proteins (19, 127). The SARS-CoV-2 S1 protein predominantly binds the protein ACE2 (35–37), although binding to 9-*O*-acetylated sialic acids (128, 129) and CD147 (cluster of differentiation 147) (130) has also been reported.

In addition to strong surface expression in alveolar epithelial cells and enterocytes of the small intestine, ACE2 is expressed by arterial and venous endothelial cells and arteriolar smooth muscle cells (126). When SARS-CoV-2 S1 binds ACE2, the spike trimer opens up and unshields the S2 core, thus facilitating fusion protein activation (131).

Henipaviruses (i.e., Nipah and Hendra virus) provide another example of viral use of a protein receptor for initial cellular attachment. Henipavirus G proteins specifically bind the vascular endothelial tyrosine kinase EphrinB2 (120, 132). Nipah virus also binds EphrinB3, which is expressed to a lesser degree on endothelial cells, but is highly expressed in the central nervous system (120). Hantaviral attachment and entry receptors are associated with more uncertainty, with β 3-integrin, gC1qR, and protocadherin-1 all being proposed as candidates, possibly with complementary roles (133). Interestingly, attachment to protocadherin-1 appears to be a clade-specific trait of new world hantaviruses. The receptor is mainly expressed on airway endothelial cells, correlating to the pathology mediated by this clade, which is the causal agent of hantavirus pulmonary syndrome (133).

Virus binding to sialic acids has been extensively studied in the context of influenza A. Sialic acids are found on the outermost end of glycan chains of most cells, but are particularly abundant in the thick luminal glycocalyx layer covering all luminal endothelial surfaces (42). Here, they maintain a negative net charge and promote the vascular barrier function (44). For influenza virus, the specificity of binding is regulated by the nature of the sialic acid linkage to underlying sugars. The ratio and distribution between 2,3-linked and 2,6-linked sialic acids in the airways vary greatly between species, and the potential for airborne transmission within specific host populations is primarily determined by whether HA preferentially targets 2,3-linked or 2,6-linked sialic acids (134, 135) [discussed in (108)]. Other viral glycoproteins attach to specific sialic acid derivatives. For example, ISAV hemagglutinin esterase (HE) exclusively binds 4-*O*-acetylated sialic acids (110, 136) expressed by endothelial, some epithelial, and red blood cells of a range of vertebrates (137). It is important to realize that while successful viral attachment is a requirement, it may not be sufficient to mediate viral infection. For example, the LPAIV strains H5N9 and H6N1 match the HA binding pattern of the endotheliotropic HPAIV H5N1 (123), suggesting that both LPAIV and HPAIV can attach to endothelial cells. Similarly, non-pathogenic epithelial-restricted and pathogenic endotheliotropic ISAV HE variants have similar receptor-binding properties (138).

Virus particles may also attach non-specifically to cells via interactions with carbohydrates and/or scavenger receptors. Such attachment strategies are particularly frequent among the flaviviruses and filoviruses that typically target a broad range of cells and appear more promiscuous in their choice of entry receptors. Subsets of endothelial cells express scavenger receptors that facilitate the removal of waste macromolecules and apoptotic cells from blood (24, 139). Stressed virus-infected cells are prone to exposing the scavenging-signal phosphatidyl serine on their outer surface, resulting in frequent exposure of phosphatidyl serine on the outside of the cell-derived viral envelopes (140).

Viral phosphatidylserines facilitate the attachment and uptake of dengue virus via scavenging receptors, either directly (TIM family receptors), or indirectly via the natural phosphatidylserine receptors GAS6 and PROS (TAM family receptor ligands) (23). Zikavirus appears to have a particularly high affinity for GAS6, providing a possible explanation for its specific ability to attach to foetal endothelial cells via the TAM receptor AXL, cross the placenta, and cause pathology in the foetus (96). Another example of a non-specific strategy is the initial attachment of ebolavirus, where uptake is facilitated by scavenger receptors like TIM-1 (141) and C-type lectins (142), the latter expressed by hepatic sinusoidal endothelial cells (142, 143). Non-specific interactions with glycosaminoglycans may also serve as a primary anchoring step that promotes subsequent engagement with a specific protein receptor. While not an emerging virus, endotheliotropic Kaposi's sarcoma herpes virus (KSHV) is a good example of this. KSHV attaches to heparan sulphate glycosaminoglycans before its subsequent specific engagement of the protein co-receptors EphrinA2 and DC-sign. Together, this attachment initiates signalling that allows fusion and cellular entry (144). DC-sign homologues are also thought to mediate the attachment of other viruses to endothelium, including dengue (145), Ebola (142), and hepatitis C virus (143).

In conclusion, viral attachment to endothelium occurs both by highly specific interactions with cellular receptors and less specific interactions with scavenging receptors or charged molecules like surface glycosaminoglycans. Binding to endothelial cell surfaces is required for successful infection and, as demonstrated by new world hantaviruses, may be linked to organ-specific pathology. Nevertheless, additional factors are needed to permit endothelial infection, as demonstrated by HPAIV and ISAV.

Access to the Endothelial Cytoplasm Relies on Viral Fusion With the Host Cell Membrane

In the next step of the infectious cycle, the virus gains access to the cellular interior. This step is essential for determining cell tropism. For enveloped viruses, viral entry happens when the viral envelope fuses with the cell membrane and releases its contents to the cytoplasm (146). Viral fusion proteins are activated in a two-step process that results in exposure of a hydrophobic fusion peptide that can be inserted into the host cell membrane to start the fusion process. This peptide may consist of mainly α -helices (class I), mainly β -sheets (class II), or a mixture of both (class III) (146). Viruses discussed here contain class I or class II fusion proteins, listed in **Table 3**.

The Availability of Relevant Proteases Regulates the Priming of the Viral Fusion Protein

Before fusion can be triggered, class I and class II fusion proteins must be primed to reach a fusion-competent state, with the possible exception of hantavirus Gc protein (147). Priming usually involves a proteolytic cleavage that may take place in different subcellular compartments or externally. Accordingly, viral fusion proteins may be primed in the Golgi

during viral biosynthesis, externally at the cell surface, or in endosomal compartments (**Figure 2C**), depending on both intrinsic properties of the fusion peptide and the range of host proteases present (146). Fusion protein priming plays an essential role in determining cellular tropism, as many proteases are expressed in a tissue-specific manner (148). For example, HA molecules of seasonal influenza virus are predominantly cleaved by airway proteases, hence, priming happens on the epithelial cell surface [discussed in (149)]. This is in contrast to avian H5 and H7 influenza viruses, where the conversion to HPAIV involves the acquisition of multiple basic amino acids at the HA cleavage site (98). This makes the site susceptible to an extended range of proteases, including ubiquitous pro-protein convertases like furin that are encountered in the Golgi apparatus during viral biosynthesis (150). Thus, susceptibility to pro-protein convertase cleavage allows the budding of virus particles that express pre-primed HA molecules and can infect a much wider range of cells than those depending on priming by tissue-restricted cell surface proteases.

A pro-protein convertase cleavage site at the S1-S2 position of the coronavirus S protein also appears to mediate extended cellular tropism. This cleavage site is present in Mediterranean respiratory syndrome coronavirus (MERS-CoV), but not in 2003 SARS-CoV. Accordingly, budding MERS-CoV viral particles express cleaved S proteins, while SARS-CoV viral particles predominantly express S proteins in the uncleaved immature conformation (151). Like MERS-CoV, SARS-CoV-2 has acquired a pro-protein convertase site at the S1-S2 junction that allows cleavage of the S protein during viral biosynthesis (35, 152, 153) and increases the efficiency of viral fusion with the plasma membrane (153). Interestingly, protein cleavage by furin may also expose a C-terminal R/KXXR/K-motif that strongly promotes cellular internalisation by interaction with the cellular receptor neuropilin-1 (154). This is also the case for SARS-CoV-2. Furin-mediated S1-S2 cleavage generates a SARS-CoV-2 S1 peptide with a C-terminal amino acid sequence (⁶⁸²RRAR⁶⁸⁵) that binds neuropilin-1, which acts as a co-factor to promote viral entry and infection (155, 156). The significance of furin cleavage to endothelial tropism has not been directly addressed, but neuropilin-1 is strongly expressed in endothelial cells in pulmonary tissues (156). Moreover, it is reasonable to hypothesise that a lesser dependency on surface-expressed proteases may extend the cellular tropism of SARS-CoV-2 and could contribute to its ability to infect endothelial cells.

Finally, the henipavirus F protein is an example of a viral fusion protein that is primed in the endosome, where it is cleaved by the endosomal protease cathepsin L (157, 158).

The Microenvironment That Triggers Fusion and the Subcellular Location Where It Is Encountered Vary Between Viruses

After priming, the triggering of fusion is initiated by a conformational change that exposes the fusion peptide (**Figure 2D**). This conformational change can happen in response to a single event, often the binding to a specific receptor or the exposure to low pH. Alternatively, it may require a series of activating events, like receptor binding followed by exposure

to low pH and/or proteolytic cleavage. Fusion proteins that depend on receptor binding only to trigger their activation, generally fuse with the host cell membrane at the cell surface (146). The previously mentioned KSHV belongs to this category, as do most other herpesviruses. This may be relevant when considering endothelial cell tropism, as some studies suggest that endothelial endosomes resist fusion of certain viruses, as discussed below.

A large proportion of enveloped viruses enters the endocytic pathway before fusion, travelling through endosomal and lysosomal compartments until they encounter a microenvironment that provides optimal conditions for triggering fusion (146). Influenzavirus HA belongs to the category of fusion proteins triggered by exposure to low pH alone. Interestingly, human lung microvascular endothelial cells show less efficient endosomal acidification than human epithelial cells (159), and this renders endothelial cells less permissive to infection by seasonal influenza virus and LPAIV strains. In addition, endothelial cells express high constitutive levels of antiviral interferon-induced transmembrane protein (IFITM) 3 in endosomal and lysosomal compartments. This protein arrests endosomal fusion of seasonal human influenza virus in endothelial cells at the hemifusion stage (160). Overcoming these obstacles, HPAIV strains have a less stable conformation of the HA molecule that allows fusion to take place at a higher pH (159) and allows infection in early endosomal compartments. The ability to efficiently fuse at a higher pH probably also mediates the relative resistance of HPAIV to the antiviral action of IFITM3 (161). Notably, it is unknown whether the inefficient endosomal acidification and high expression of IFITM3 are specific to the lung microvasculature or a general feature of endothelial cells. The relevance of these findings to other anatomical sites is therefore unclear. Furthermore, the efficiency with which IFITM proteins inhibit viral entry vary between viruses. Flaviviruses, represented by dengue and West Nile virus, show a similar susceptibility to IFITM proteins as influenza H1N1, while arenaviruses appear to overcome their action (162).

In viruses where separate viral glycoproteins mediate cellular binding and fusion, interactions between these proteins may also regulate viral entry. This has been extensively studied in paramyxoviruses, where three different models have been proposed: First, the *provocateur model*, where viral binding to cellular receptors, often sialic acids, triggers an activating interaction between the viral attachment and fusion proteins; Second, the *clamp model*, where the interaction between the viral attachment and fusion proteins inhibits the fusion protein; And third, the *safety-catch model*, where the fusion protein head tightly assembles with the stalk protein of the attachment viral glycoprotein in the endoplasmic reticulum during viral bio-synthesis, but is released at the target cell membrane upon receptor binding (163). A highly relevant example of the safety-catch model is the binding of endotheliotropic henipaviruses, where binding of the globular head of dimeric G-proteins to their cellular receptors (EphrinB2 and B3) mediates a distant structural change that releases the association with the F-protein, thus allowing fusion to be triggered (163).

The ISAV fusion process is still incompletely characterised, but appears to have features in common with both influenza and paramyxoviruses. For the F protein to reach its fusion-competent trimeric state, both low pH and proteolytic cleavage is required (164). The conversion to virulence that includes a gain of endotheliotropism, always involves deletion of a variable number of amino acids in a highly polymorphic region [HPR, including amino acids 320–374 (165)] at the base of the stalk of the hemagglutinin esterase molecule (166, 167). In apparent homology to the destabilisation of influenza HA that is associated with the conversion of LPAIV to HPAIV, the shortening of the stalk that results from HPR-deletion destabilises the interaction with the fusion protein. This destabilisation appears to facilitate the dissociation of HE and F proteins upon receptor binding and allow activation of viral fusion at neutral pH (166, 168). In contrast, no increase in fusion efficiency was observed when comparing pathogenic and non-pathogenic variants of the HE and F proteins in the presence of the exogenous protease trypsin and low pH (168), perhaps mimicking the situation at mucosal surfaces.

Exposure of the fusion peptide by receptor binding followed by protease cleavage, sometimes with the added requirement for low pH, has been studied extensively in coronaviruses and is therefore of great relevance to the current pandemic. Endothelial cells express a wide range of proteases on their luminal surface that take part in activation of surface receptors with homeostatic functions. As discussed above, the SARS-CoV-2 protein is cleaved during biosynthesis by pro-protein convertases. However, further S-protein processing occurs at the target cell surface by the protease TMPRSS2 and in target cell lysosomes by cathepsin, and the three proteases appear to have cumulative effects in regulating the entry of SARS-CoV-2 (153). In contrast to cathepsins that are ubiquitously expressed in lysosomes, TMPRSS2 expression differs between tissues and has been proposed to restrict the target cell population of SARS-CoV-2 (169). While TMPRSS2 is strongly expressed by human endothelial cells in culture (124), only a minor fraction of tissue endothelial cells appears to express the TMPRSS2 gene under basal conditions (125, 170, 171), perhaps limiting endothelial SARS-CoV-2 infection. Yet, endothelial cells are infected (2, 3), presumably assisted by the additive action of pro-protein convertases and cathepsins.

The composition of the host cell membrane may also regulate the potential for viral fusion. A prime example is the Andes hantavirus that shows a strict requirement for membrane cholesterol that is shared by several other pathogenic hantaviruses (172). Similarly, dengue virus requires anionic lipids in the host cell membrane and therefore fuse in later endosomal compartments than their pH-requirements would indicate (173).

Replication in Endothelial Cells Requires Evasion of Intrinsic Antiviral Responses

Once inside the cell, efficient viral replication depends on adaptation to the host cell machinery. The ability of viral polymerase complexes to function efficiently in the cellular interior is a key factor for determining species tropism and

virulence, as demonstrated for PB2 and PA subunits of influenza (174, 175). Moreover, viral polymerase subunits must be able to associate with the proteins that mediate nuclear import in the cell type in question (176). Nevertheless, successful viral adaptation to the endothelial cell type may rely even more on the ability to evade intracellular antiviral responses that vary between cell types. For example, replication of pathogenic hantavirus was sustained in cultured endothelial cells for up to 48 h, while infection with a non-pathogenic strain resulted in rapid upregulation of antiviral proteins that prevented efficient generation of infective progeny (177). This correlates to observations in ISAV infection, where a pathogenic isolate of low virulence infected endothelial cells early in the course of infection, but was rapidly eliminated, while a high-virulent pathogenic isolate that caused a less pronounced antiviral response, maintained replication in endothelial cells over a prolonged period of time (178, 179). In conclusion, the efficiency of viral replication in any cell type relies on successful viral adaptation to the cellular replication machinery as well as the ability to evade intrinsic antiviral responses.

THE ENDOTHELIAL CELL RESPONSE TO VIRAL INFECTION

Cellular Detection of Viral Components Activates NF- κ B and Type I Interferon Signalling

All cells are capable of intrinsic antiviral responses. First, cellular pattern recognition receptors at different subcellular sites detect the presence of viral nucleic acids. The Toll-like receptors TLR7 and TLR8 (both detecting RNA), and TLR9 (detecting DNA) bind viral nucleotides on cell surfaces or in endosomes. Once released to the cell interior, viral contents can also be detected by the cytoplasmic receptors RIG-like (e.g., retinoic acid-inducible gene-I, RIG-I, detecting single stranded RNA), melanoma differentiation-associated protein 5 (MDA5, detecting double stranded RNA), and cyclic GMP-AMP synthase (cGAS, detecting DNA). Recent work suggests that the surface-expressed receptor TLR4 can also be activated by secreted viral proteins (92, 180). Stimulation of these receptors initiates signalling that results in the intracellular activation of inflammatory (NF- κ B) and type I interferon (interferon response factors 3 and 7) transcription factors (181). Similar to other cell types, endothelial cells mount type I interferon responses upon viral infection (177, 182). This response stimulates the transcription of a range of interferon-stimulated genes (ISG) that inhibit viral infection (183). Furthermore, endothelial activation of NF- κ B results in upregulation of a range of molecules that mediate leukocyte adhesion and shift the endothelial surface toward a pro-thrombotic stage (184) (**Figure 1C**).

Inflammatory and Antiviral Signalling Is Also Activated in Non-infected Cells

Non-infected cells, including endothelial cells, also respond to viral infection (104). Endothelial cells express receptors for type I and II interferons, as well as a range of cytokines and chemokines upregulated in and secreted from virus-infected

cells or activated immune cells (**Figure 1C**). Such activation is highly prevalent in patients with H1N1 2009 pandemic influenza and COVID-19 (81, 90). Another example of such humoral activation is the vasoactive proteins secreted by dendritic cells and macrophages in ebolavirus infection (180). In addition, non-infected cells can be activated in viral infection by diffusion of second messengers across intercellular gap junctions (185). Although only demonstrated for the cGAS second messenger cGAMP(2',5'), it is likely that other low molecular weight (<1,000 Dalton) second messengers also share this route (186). Gap junction-mediated activation of endothelial cells has not been explored in the context of viral infection. However, the mechanisms could be highly relevant, as endothelial cells form gap junctions with other endothelial cells, vascular smooth muscle cells, and pericytes that contribute to the maintenance of vascular homeostasis (68, 69).

Endothelial Cells Are Effectors of the Host Response to Viral Infection

Endothelial NF- κ B activation is capable of mediating all cardinal signs of inflammation. Redness, swelling, heat, and pain result from an increase in local blood flow, leakage of protein-rich plasma, and the recruitment of leukocytes that release mediators working on C-type sensory nerve fibres. Invading leukocytes attempt to eliminate the initiating stimulus by killing infected cells and removing cellular debris. However, the powerful inflammatory mediators released by leukocytes also exacerbate vascular activation, cause tissue damage, and loss of function (**Figures 1C,D**). The first cells recruited to sites of inflammation are neutrophils that infiltrate tissues within minutes after endothelial type I activation (53). Nevertheless, a recent study showed that recruitment of non-conventional CD8 $\alpha\beta$ -T cells from the microvasculature occurred with similar ultra-rapid dynamics when a rhabdovirus was inoculated in the nasal cavity of trout, and that this recruitment was central to controlling infection (187). The mechanisms involved are not yet fully understood, but appear to depend on viral activation of tyrosine kinase receptors (187). Altogether, the dynamic vascular expression of adhesion molecules and chemokines in inflammation contributes to tight coordination of the leukocyte recruitment process. Such control is particularly relevant in viral infection, where pathology often arises from the host response to infection, rather than the action of the virus itself. The importance of controlling early innate responses and preventing their spiralling out of control is clearly demonstrated in COVID-19, where an increased neutrophil-lymphocyte ratio is a feature of severe disease and has emerged as a prognostic indicator of outcome (188).

Endothelial Cells Modulate Immune Cell Activation in Viral Infection

Infection with some endotheliotropic viruses, like SARS-CoV-2 (2) and Nipah (9, 189), is associated with extensive endothelial damage and endotheliitis (**Table 1**). In contrast, other endotheliotropic infections are characterised by a lack of perivascular cellular infiltrates and/or endothelial cytopathology (11, 13). This suggests suppression of cytotoxic T cell responses, which would otherwise attempt to limit infection

by killing infected cells. In this context, it is interesting to consider the immunomodulatory potential of antigen-presenting endothelial cells. As part of protective measures against self-reactive T cell responses, interferon-induced MHC class II allows endothelial cells to act as semi-professional antigen-presenting cells (190). Importantly, type I interferon signalling also upregulates programmed cell death 1 (PD-1) ligand (PD-L1) in endothelial cells (191), and T cell activation by PD-L1-bearing lymphatic endothelium induces accumulation of PD-1 and T cell deletion (192). Hence, endothelial viral antigen presentation is likely to promote immune tolerance to protect vascular integrity, but also provide a niche for viral evasion of immune responses (41). Interestingly, PD-L1 on both haematopoietic and non-haematopoietic cells reduced viral clearance in experimental LMCV infection, yet improved survival by reducing immunopathology (193). There has been a recent discussion about the possible impact of PD-1 blockade, a commonly used cancer immunotherapeutic, on SARS-CoV-2 infection. Nevertheless, current clinical data suggest that it does not change the outcome in COVID-19 (194).

MOLECULAR MECHANISMS THAT DISRUPT VASCULAR FUNCTION IN VIRAL INFECTION

Maintaining a Stable Vasculature Is Key to Preventing Viral Pathology

Studies of the S1P-receptor illustrate the central role endothelial cells have in maintaining the vascular barrier and preventing detrimental inflammation. Activation of endothelial S1P-signalling by a synthetic agonist suppressed early innate immune responses and improved survival in mice infected with H1N1 2009 pandemic influenza virus (83). S1P-administration also ameliorated hantavirus-induced endothelial hyper-responsiveness in a cell-based permeability assay (195), showing that S1P-signalling has a generalised stabilising effect on the vasculature. Underscoring the importance of endothelial signalling in maintaining a functional vasculature, mice with endothelial-specific deletion of the glucocorticoid receptor showed increased mortality in systemic inflammatory response syndrome, with exaggerated vascular instability and pro-inflammatory signalling (196). This makes it tempting to speculate if some of the positive effects of glucocorticoid-treatment in severe COVID-19 (197) could be mediated by vascular stabilisation. An overview of the molecular mechanisms that regulate the stability of vascular junctions in viral disease is provided in **Figure 3**.

Disruption of Vascular Function by Host Defence Mechanisms

Excessive NF- κ B and Type I Interferon Signalling Disrupts Endothelial Function

Signalling that confers powerful antiviral actions also has less beneficial effects (**Figure 1**). Type I interferon responses are associated with endothelial dysfunction in systemic lupus erythematosus (198). They also accelerate the development of

atherosclerosis in both mice and humans, at least in part by reducing NO bioavailability [reviewed in (199)]. Finally, type I interferon skews the profile of endothelial-secreted molecules toward a pro-thrombotic state and inhibit normal angiogenic functions that may be relevant to reparatory processes (200). Many ISGs have dual, counteractive functions, a highly relevant example being PAI-I (201). PAI-I is an extracellular serine protease inhibitor that counteracts viral entry by inhibiting tryptase, trypsin, and TMPRSS2, thereby inhibiting priming of influenza virus HA (201). However, PAI-I is best known for its pro-thrombotic actions and is a biomarker for endothelial dysfunction (202). Hence, excessive or prolonged endothelial secretion of PAI-I will disturb the delicate maintenance of blood fluidity (**Figure 1D**).

Similarly, excessive endothelial NF- κ B-activation is linked to the disruption of normal vascular function. Stimulation of NF- κ B promotes a pro-inflammatory and pro-thrombotic endothelial phenotype (184) and inhibits NO production (203). Accordingly, endothelial-specific deletion or inhibition of NF- κ B protects mice from developing atherosclerosis (204) and abrogates the derangement of vascular functions in experimental sepsis (203). In conclusion, prolonged or excessive endothelial activation in viral disease is likely to result in vascular dysfunction that affects both infected and bystander endothelial cells.

Mast Cell Activation Induces Vascular Hyper-Permeability

Host cells may also secrete other vasoactive molecules. One of many examples is the vascular hyper-permeability induced by vessel-associated mast cells in dengue-infection, involving tryptase-mediated breakdown of intercellular endothelial junctions (205). Tryptase levels correlated with the severity of symptoms in two patient cohorts (205), supporting that this mechanism is relevant to disease pathogenesis.

Disruption of Vascular Function by Viral Components

Viral Surface Proteins That Dysregulate Vascular Homeostasis

Many of the cellular molecules that viruses bind (**Table 2**) are also central to the regulation of vascular function (209). Hence, viral infection has potential to dysregulate their signalling. In the COVID-19 pandemic, the dual roles of ACE2 have been subject to much attention (19, 210), on one hand serving as a key factor for viral attachment and entry (153), and on the other hand, counteracting the detrimental cardiovascular effects of ANG-II (19, 38), highly relevant to disease. Interestingly, internalisation of the closely related SARS-CoV is accompanied by internalisation and shedding of ACE2 (211, 212). By homology, it is broadly assumed that the same is true for SARS-CoV-2 (19, 210, 213). The resulting loss of the ACE2 catalytic effect at the cell membrane may be a significant contributor to pathology in COVID-19 (211, 213). Briefly, such downregulation of ACE2 favours the progression of inflammatory and thrombotic

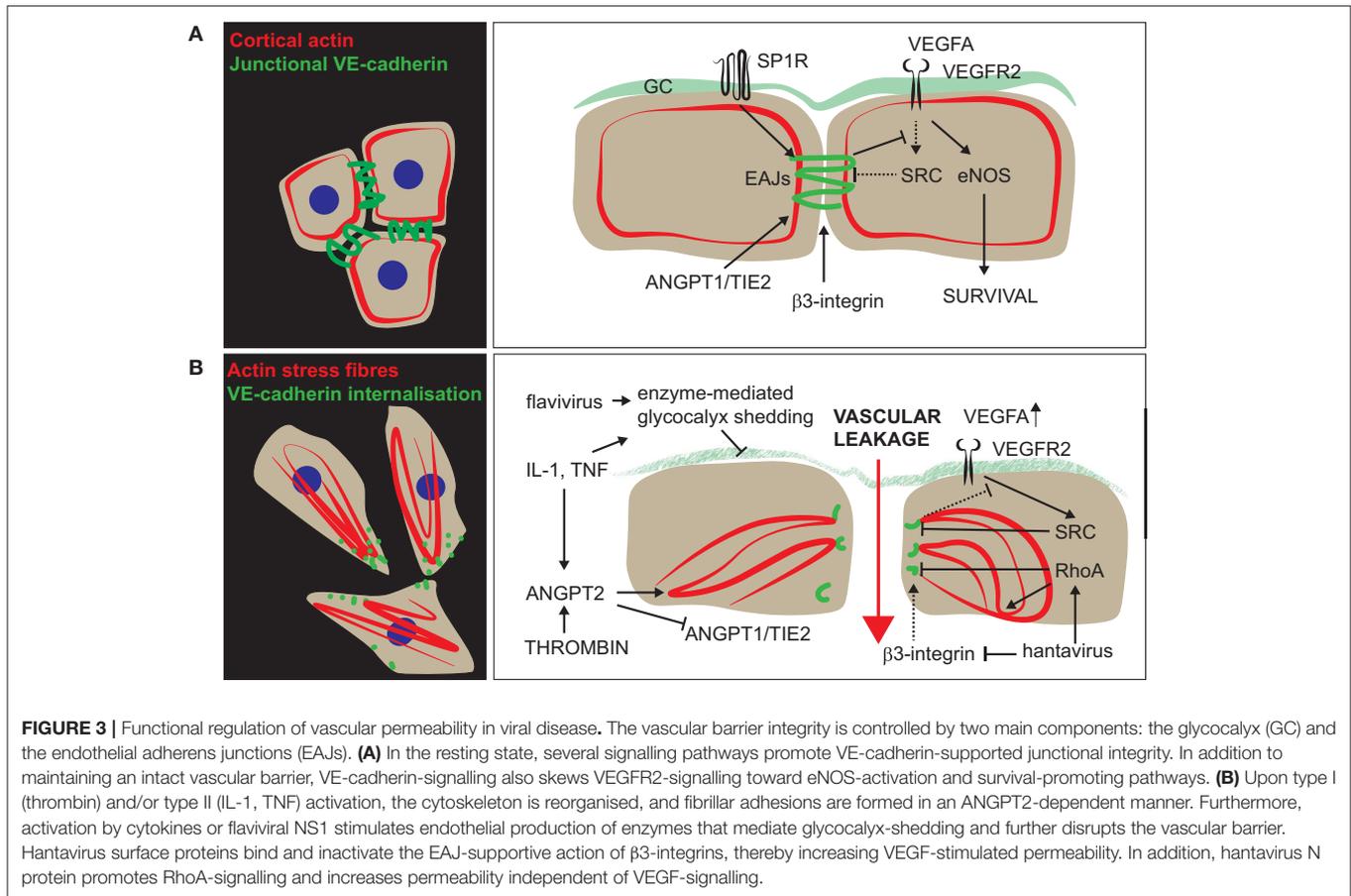


TABLE 2 | Viral attachment molecules that bind endothelial surface molecules.

Virus	Viral attachment protein	Endothelial receptor	References
SARS-CoV-2	S protein, subunit S1	Angiotensin converting enzyme (ACE)-2	(35–37)
Nipah and Hendra henipavirus	G protein	EphrinB2 (B3)	(120, 132)
New world hantavirus	Gn protein	β 3-integrin protocadherin-1	(133)
Influenza A, H5N1, H7N1 (HPAIV)	HA, subunit H1	α -2-3-linked N-acetyl-sialic acid	(134, 135)
Infectious salmon anaemia virus (HPR-deleted)	HE	4-O-acetylated N-acetyl-sialic acid	(110, 206)

processes, triggered by enhanced and unopposed effects of ANG-II (19, 38, 213).

Another highly interesting SARS-CoV-2 interaction partner is the VEGFA- and semaphorin-binding b1 pocket of neuropilin-1 (155, 156). To our knowledge, the effects of this interaction on vascular permeability has not yet been evaluated in the context of SARS-CoV-2. Nevertheless, binding of C-end R/KAAR/K-peptides to this pocket generally increases vascular permeability in a VEGFR2-independent manner (214), and, upon injection, such peptides induce vascular leakage and are retained in tissues (154).

Another example of viral interactions with the VEGFR2-signalling pathway is the dysregulation of integrins in hantavirus pulmonary syndrome (**Figure 3**). Integrins have central functions

in maintaining vascular barrier functions (215, 216), and β 3-integrins specifically regulate VEGFR2-dependent vascular permeability (217). Pathogenic hantaviruses bind α β 3 integrin in the bent, inactive conformation (218). The resulting reduction in α β 3 integrin signalling results in VE-cadherin internalisation and increased vascular responsiveness to VEGF-A (195), mimicking the actions of α β 3 integrin antagonism or genetic silencing of β 3 integrin (57, 219) (**Figure 3B**).

EphrinB2, the cellular interaction partner for the henipavirus G protein (132), also modulates VEGFR2-signalling (220–222). Ephrins and Ephs are membrane-bound tyrosine kinases that signal bidirectionally. This leads to forward (in the Eph-bearing cell) and reverse (in the Ephrin-bearing cell) signalling in adjacent endothelial cells (223). EphrinB2-Eph4

TABLE 3 | Viral fusion proteins and factors that regulate their activation.

Virus	Protein containing fusion peptide	Priming proteolytic cleavage (site)	Triggering	References
SARS-CoV-2	S protein (S2 subunit)	Cleavage of S1 and S2 subunits that remain non-covalently bound (surface or endosome)	Proteolytic cleavage at the S ₂ ' site, immediately upstream of the fusion peptide	(35)
Nipah and Hendra henipavirus	F protein	Cleavage of F0 by cathepsin into F1 and F2 (endosome)	G protein receptor binding that mediates dissociation of the F protein	(157, 158, 163, 207)
New world hantavirus	Gc protein	Incompletely understood	Low pH, membrane cholesterol composition	(172)
Influenza A, H5N1, H7N1 (HPAIV)	HA, subunit H1	HA0 cleaved to HA1 (receptor binding) and HA2 (fusion), subunits remain non-covalently bound. (cell surface, endosome, Golgi)	Low pH	
Infectious salmon anaemia virus (HPR-deleted)	F protein	Cleavage of F0 by unknown protease into F1 and F2 (location not known)	Low pH, not fully characterised	(164, 208)

signalling appears to support endothelial-pericyte interactions after cerebral ischaemic injury and promote the return to a functional vasculature (224). While EphrinB2-Eph4 signalling is dispensable for blood-endothelial barrier function, it is a critical stabiliser of lymphatic endothelial junction integrity (225). Therefore, it is tempting to speculate if viral EphrinB2 binding and internalisation could modulate EphrinB2-Eph4 signalling in henipavirus-infected endothelial cells. However, to our knowledge this has not been experimentally tested. The surface proteins of henipaviruses also mediate another central vasculopathy, namely the endothelial syncytia formation observed in both human (9) and hamster (189), a species commonly used to study the disease. This is reflected in cultured cells, where co-expression of henipavirus G and F proteins induces cellular fusion within 12 h (226).

Viral attachment to sialic acids also has the potential to affect vascular functions. In several viral infections, including influenza, a reduction in virus particle binding to host tissues is observed as the infection progresses (227), limiting superinfection (228). The mechanism behind the loss of virus binding is not fully characterised, but several studies suggest that sialic acid cleavage by viral neuraminidase may be involved (228–230). This is interesting, as sialic acids in the endothelial glycocalyx contribute to maintaining vascular integrity, and their removal has been associated with increased microvascular permeability (44). However, the consequences of virus-mediated sialic acid modulations on vascular function remain largely unknown.

Secreted Viral Proteins Damage Vascular Endothelial Cells Independent of Their Infection Status

Another virus family that targets the endothelial glycocalyx is the flavivirus family. An extensively studied example is the dengue virus-mediated vascular barrier breakdown that involves disruption of glycocalyx integrity in cell culture and animal models (231, 232). The damage is mediated by the secreted non-structural viral protein NS1. Dengue NS1 directly induces shedding of the pulmonary endothelial glycocalyx by upregulation of endothelial sialidases, cathepsin L, and heparanases (232) (**Figure 3B**). In fact, NS1 proteins of different

flavivirus strains selectively target the endothelium of specific vascular beds in a pattern that corresponds to virus tropism and causes disruption of the endothelial glycocalyx and severe vascular leakage (119). At least for dengue virus, the capillary leak induced by NS1 is reduced by inhibition of TLR4 (92), suggesting that this receptor mediates the response to NS1. Interestingly, soluble viral glycoproteins appears to counteract the disruption of the vascular barrier caused by particle-bound viral glycoproteins in Ebola virus infection (233). Notably, infection of endothelial cells mostly occurs after clinical signs have developed in natural Ebola infection (16). Hence, most vascular damage is probably indirect, for example by the action of vasoactive cytokines secreted by dendritic cells and macrophages (180).

Modulation of Endothelial Signalling by Other Viral Proteins

The Andes hantavirus nucleocapsid (N) protein mediates an additional aspect of the increased microvascular permeability observed in hantavirus pulmonary syndrome (84). In contrast to disrupted integrin signalling that targets VEGF-stimulated vascular permeability (195), the N protein increases endothelial cell size by interfering with tuberous sclerosis complex inhibition of mTOR signalling (84). Because tuberous sclerosis complexes also regulate RhoA-signalling, the inhibition also activates RhoA and increases basal (unstimulated) vascular permeability (84) (**Figure 3B**).

In conclusion, both structural and non-structural viral proteins modulate and disrupt endothelial function at different stages of the infectious cycle. Importantly, cellular damage can be induced by secreted viral proteins, hence, uninfected endothelial cells can also be affected.

CONCLUDING REMARKS

We have reviewed endothelial cell-virus interactions in emerging viral infections. We aimed to illustrate the diversity of such responses and identify common features that will bring us closer to understanding the role of endothelial cells in viral disease. This

is important. First, because many pathogens that pose a threat to global human and animal health infect endothelium; second, because conditions characterised by endothelial dysfunction is associated with severe outcomes of infection with SARS-CoV-2; and third, because dysregulation of vascular function confers disease in many life-threatening viral infections.

All possible viral entry ports are in close contact with endothelial cells, and the diversity of endothelial phenotypes probably contributes to guiding viral infection and pathology. In addition, the switch from a non-pathogenic to a virulent phenotype that causes disseminated infection and generalised disease is associated with a gain in endotheliotropism, at least for some viruses. This endothelial tropism is determined by both virus and host factors that regulate virus attachment, fusion, and replication. The viral attachment has a prominent role in species- and tissue-tropism but is not sufficient for infection to occur. Rather, the viral fusion process appears to be a strong determinant of cellular tropism. Some essential features of endothelial cells that regulate such viral fusion include inefficient endosomal acidification and high levels of antiviral proteins in endosomal compartments (159, 161). Hence, an increased ability to fuse on the cellular surface or in early endosomal compartments may facilitate, but is not absolutely required, for endothelial infection. This may for example be achieved by conformational destabilisation of the fusion protein that makes it more susceptible to activation (159, 168). Much of the work that has identified specific factors regulating endothelial tropism comes from the study of orthomyxoviruses. Hence, efforts should be made to understand if these factors also determine endotheliotropism in other viruses. Studies of viral fusion are challenging in other settings than cell culture, but should be interpreted with a view to natural infections.

A systemic inflammatory response syndrome with the breakdown of vascular function characterises severe viral disease, and it has clear parallels to bacterial septic shock (89). There is a loss of control of vascular permeability, ranging from local oedema to severe hypovolemic shock, and a microvascular thrombosis that may proceed to consumptive coagulopathy. Despite the similarity of clinical signs observed in infection by different endotheliotropic viruses, the underlying disease mechanisms differ. For example, the extent of direct endothelial damage and endotheliitis vary widely, from extensive in henipavirus and SARS-CoV-2 infection (2, 3, 9) to minimal in hantavirus (11, 41) and ISAV (13) infection. Nevertheless, all these agents cause severe vascular compromise, oedema, and bleeding. This has two implications. First, mechanisms other than direct endothelial damage may cause vascular dysfunction in viral disease. Second, the immunomodulatory role of endothelium

probably limits T cell killing of infected endothelial cells in some viral infections, possibly providing a niche for viral replication (192). Activation of inflammatory and antiviral pathways in endothelial cells orchestrate leukocyte recruitment to sites of infection and generate ISGs that potentially inhibit viral replication. However, the same pathways also disrupt vascular function (33, 204, 234) by inducing a pro-inflammatory, pro-thrombotic vascular profile. In addition, direct interactions between viral proteins and endothelial components that regulate the vascular barrier contributes to the vascular leakage seen in viral disease (84, 92, 119, 195, 227, 228).

The biosecurity risks associated with many emerging viruses together with the invasiveness of sampling required for obtaining endothelial cells from patients, limit studies of viruses during natural human infection. However, all available model systems have significant limitations. To this end, we propose that experiments in cell and laboratory animal models, studies of animal pathogens in their natural hosts, and analysis of patient materials must be combined to fully understand viral interactions with the endothelium during infection and disease.

We hope that this article is a step toward a more generalised understanding of how endothelial cells are targeted in viral disease, either by direct infection or as bystanders. Such knowledge is likely to improve strategies for managing patients with viral infection. This is particularly important in crises like the current pandemic, where resources are exhausted by the high prevalence of infection. A better understanding of underlying mechanisms could help us evaluate the rationale of suggested strategies. One example is the stratification of risk in patients by FMD in COVID-19 (7), another the use of pleiomorphic therapies, including statins, glucocorticoids, and ACE-inhibitors, to stabilise vascular function in a non-specific manner in severe viral infection (235, 236).

AUTHOR CONTRIBUTIONS

JF: Concept. JF and RE: First draft. JF, RE, KF, and GH: Developing manuscript into final version. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: KF was employed by the company AquaMed Consulting AS.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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