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Virus Interactions with Endothelial Cell Receptors: Implications for Viral Pathogenesis

Nadine A. Dalrymple and **Erich R. Mackow**
Stony Brook University

Abstract

The endothelial lining of the vasculature performs a vital role in maintaining fluid barrier functions despite balancing nutrient and fluid content of tissues, repairing localized damage, coordinating responses of a plethora of factors, immune cells and platelets through a multitude of endothelial cell surface receptors. Viruses that nonlytically cause lethal hemorrhagic or edematous diseases engage receptors on vascular and lymphatic endothelial cells, altering normal cellular responses that control capillary leakage and fluid clearance functions with lethal consequences. Recent studies indicate that receptors directing dengue virus and hantavirus infection of the endothelium contribute to the dysregulation of normal endothelial cell signaling responses that control capillary permeability and immune responses that contribute to pathogenesis. Here we present recent studies of virally altered endothelial functions that provide new insight into targeting barrier functions of the endothelium as a potential therapeutic approach.

Introduction

The endothelium is a tissue that lines capillaries and regulates solute, gas, and fluid exchange between tissues and vascular compartments through a complex series of endothelial cell (EC) surface receptor interactions [1, 2]. The critical nature of the EC fluid barrier is evident from the redundant failsafe mechanisms in place to prevent a lethal vascular breach and a discrete lymphatic system designed to clear excess fluid from interstitial spaces [3]. Microvascular and lymphatic EC (MEC and LEC) surface receptors and the endothelial glycocalyx are keys to fluid management and vascular homeostasis. The endothelial glycocalyx is mainly composed of surface-anchored syndecans and glypicans carrying highly sulfated, linear glycosaminoglycan attachments such as heparan, chondroitin, and dermatan [4]. Interactions between the glycocaylx, cell surface integrins, (ie. $\alpha_v\beta_3$, $\alpha_v\beta_1$) adhesions molecules (ie. PECAM, ICAM, and VCAM), and inter-endothelial adherens junctions (VE-cadherin) form a meshwork of EC specific cell surface sensors that maintain EC barrier functions [4]. This task is complicated by the requirement

*Corresponding Author: Erich R. Mackow, Ph.D., Dept. of Molecular Genetics and Microbiology, Stony Brook University, Life Sciences Bldg. Rm. 126, Stony Brook, NY 11794-5222, Phone: 631-632-7014, Erich.Mackow@stonybrook.edu.

for ECs to respond to a plethora of permeabilizing factors (ie. VEGF, TNFα, PAF), tissue conditions, damage responses, and immune cell extravasation that require junctional plasticity while maintaining a fluid barrier.

Although the endothelium normally prevents adhesion of leukocytes and platelets, pathogen activation of the endothelium directs localized immune cell adherence and extravasation without EC lysis or hemorrhage [4–12]. However, localized concentrations of cytokines, chemokines, clotting cascades, growth factors, and nitric oxide, whose concentrations are increased as a result of infection, may engage EC receptors and reduce barrier integrity [1, 2, 13–18]. Inflammatory mediators such as TNFα and LPS can also cause degradation or shedding of the EC glycocalyx [4]. TNFα induces EC activation, attracting mast cells and inducing responses of cytokines, proteases, and heparanases that degrade glycocalyx moieties and glycan receptors [4, 19].

Altered endothelial barrier functions are implicated as the cause of hemorrhagic disease following infection by a number of viruses, including dengue viruses, hantaviruses and arenaviruses, that nonlytically infect ECs [5–12, 20]. Changes in EC functions are likely to result from EC surface receptor and cytoplasmic signaling responses as well as EC interactions with immune cells. Dengue viruses engage EC surfaces through interactions with heparan sulfate moieties that direct viral entry [21]. Dengue virus infection of ECs results in changes in signaling pathways and cellular gene expression profiles, which in turn may influence EC fluid barrier functions both directly and through the induction and secretion of immune-enhancing chemokine and cytokine responses [21, 22]. Thus the means by which dengue attaches to and enters ECs is central to its ability to direct disease and fundamental to therapeutically resolving dengue-induced vascular permeability deficits.

Direct contact with EC surface receptors is also associated with changes in vascular permeability via signaling pathway responses resulting in the dissociation of VE-cadherin within adherens junctions (AJs) [23–26]. Under normal conditions VEGF directs the dissociation of AJs in order to repair vascular damage. However, VEGF is also 50,000x more potent than histamine in directing EC permeability, and high altitude induced pulmonary edema is the result of aberrant hypoxia-induced VEGF permeability [13, 14, 17, 26–29]. Hantaviruses bind and inactivate $\alpha_v\beta_3$ integrin conformers that normally form complexes with VEGF receptors, and thus hantaviruses similarly disengage the normal regulation of VEGF-induced permeability [7, 30–37].

The endothelium contains a vast array of receptors that play critical roles in the regulation of immune cell adherence, capillary permeability, platelet and complement activation, clotting, and vasodilation responses, all of which can be greatly altered by virus infection and contribute to hemorrhage or edema [10]. In addition, lymphatic tissues and lymphatic endothelial cells (LECs) are uniquely regulated by discrete cell surface receptors and emerging as a system critical to the regulation of edema, tolerance, and immunity [28, 38–40]. Although lymphatics are often forgotten, these vessels clear fluid from tissues and in lymph nodes LECs constitutively express MHCII and are sentinel antigen presenting cells that determine tolerance and clearance responses [38, 41]. Recent studies indicating that hantaviruses infect and dysregulate normal LEC functions further suggest roles for

lymphatic EC receptor responses that alter pulmonary fluid clearance functions of hantavirus pulmonary syndrome (HPS) patients [39, 42, 43]. This review explores the ways in which dengue and hantaviruses contact and alter endothelial cell surface receptors and their corresponding signaling pathways, leading to hemorrhagic disease and vascular permeability.

Endothelial Cells and Dengue Virus

Dengue virus is a mosquito-borne flavivirus that causes a mild febrile illness, dengue fever (DF), and two highly lethal vascular permeability based diseases: dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [44, 45]. DSS and DHF are edematous and hemorrhagic diseases, respectively, that occur in the absence of EC lysis [45]. The presence of dengue virus infected ECs in patients rationalizes their contribution to severe dengue disease [5, 46–49], and while their role in pathogenesis is still unknown, infected ECs can potentially alter barrier functions, permit immune cell targeting, elicit cytokine and chemokine responses, and contribute to viremia [50, 51]. Dengue virus reportedly infects a variety of cells including immune, dendritic, endothelial, and liver cells through attachment to cell surface receptors. The dengue virus envelope protein reportedly binds to Fc receptors, DC-SIGN, ICAM3, CD14, mannose receptor, HSP70/90, GRP78, laminin receptor, heparan sulfate proteoglycans (HSPGs), and the mannose receptor [52–62]. However, a consensus dengue virus receptor has not yet been defined.

Recent studies show that dengue virus infects ~80% of primary human ECs with viral antigen present by 24 hours after infection. Infection is rapidly productive, releasing ~10^5 FFUs/ml of dengue virus into the media 1 day post-infection [21]. Furthermore, heparin, heparan sulfate, heparinase and protease, but not antibodies to a number of other cell surface receptors, block dengue virus infection of primary human ECs. Dengue virus binds specifically to immobilized heparin and is competitively inhibited by the addition of excess heparin [21]. Thus, dengue virus productively infects human ECs via attachment to heparan sulfate-containing cell surface receptors. Indeed, carbohydrate moieties of cell surface glycoproteins, glycolipids, and proteoglycans serve as receptors for enveloped and non-enveloped viruses alike [63]. These negatively-charged carbohydrate receptors are also commonly responsible for specific tissue tropism, making them key targets for limiting viral spread [63].

Dengue nonstructural proteins may also enhance DV pathogenesis through a variety of cytoplasmic and cell surface receptor directed signaling mechanisms. In particular, the non-structural 1 (NS1) protein is expressed in cytosolic, secreted, and cell-surface expressed forms [64–66]. Secreted NS1 is highly abundant, highly antigenic, and shown to bind cellular heparan sulfate E present on liver and lung ECs [67]. Likewise, high quantities of adherent cross-reactive NS1 antibodies circulate in infected patient blood and are known to bind glycan surface receptors on platelets and ECs [68–70], triggering immune cell and complement activation [71, 72]. Furthermore, the secreted form of dengue NS1 also modulates classical complement activation by binding to the C4b binding protein, thereby inactivating C4b [72]. NS1 and NS1 antibodies form a potent combination capable of eliciting or regulating immune and complement responses through critical cell surface

glycan interactions [73]. Interestingly, dengue virus infected ECs elicit immune enhancing cytokine and chemokine responses that may enhance immune responses and contribute to DSS and DHF diseases during secondary infections perhaps by targeting non-neutralizing dengue antigens in ECs. These interactions, and the intracellular signaling responses they trigger, may contribute to EC dysfunction and vascular leakage in dengue-infected patients [5, 68–70]. The differential role of dengue virus regulation of EC MHCII responses in primary and secondary dengue virus infections has yet to be considered but may also factor into pathogenic mechanisms during infection by a second dengue serotype. Consideration of barrier-stabilizing effectors that target the endothelium may also effectively reduce vascular leakage and associated inflammatory effects that contribute to dengue pathogenesis [74, 75].

Hantavirus Endothelial Cell Interactions

Diverse pathogenic hantaviruses that cause hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS) were found to commonly use $\alpha_v\beta_3$ integrins to enter primary human ECs or CHO cells expressing recombinant human $\alpha_v\beta_3$ receptors [34, 76–79]. Interestingly, nonpathogenic hantaviruses failed to use $\alpha_v\beta_3$ integrins and instead entered cells consistent with the use of β1 integrins, suggesting a fundamental difference in EC receptor usage that is tied to vascular permeability [34, 76]. $\alpha_v\beta_3$ integrin deficits cause vascular diseases and subsequent studies found that pathogenic hantaviruses bind inactive $\alpha_v\beta_3$ integrin conformers [34, 76, 78, 79]. $\alpha_v\beta_3$ integrins normally regulate permeabilizing responses of VEGF directed by VEGFR2 receptors and pathogenic hantavirus infections cause the hyperpermeability of ECs in response to VEGF days after infection [77, 80, 81]. These responses are mediated by increased VEGFR2 phosphorylation and increased internalization of VE-cadherin from AJs and suppressed by blocking VEGFR2-Src signaling pathways. The occurrence of these responses days after viral entry suggests that newly emergent cell-associated virus regulates $\alpha_v\beta_3$ responses (Figure 1). In fact pathogenic hantavirus accumulation on the EC surface was shown to occur through $\alpha_v\beta_3$ interactions and to recruit quiescent platelets to ECs [80]. These findings define EC receptors as targets of dysregulated VEGF-directed permeability responses and potential mechanisms by which hantaviruses inactivate platelets and contribute to thrombocytopenia. They also suggest quiescent platelet recruitment to infected ECs as a means of evading immune surveillance.

HPS patients are acutely hypoxic and hypoxia is a known inducer of VEGF directed permeability and edema. Similar to patient responses, hantaviruses enhance the permeability of chemically- or O_2 level-induced hypoxia in MECs and LECs. This constitutively activates a downstream mTOR-directed pathway that normally regulates hypoxic responses, VEGF signaling and cellular quiescence [42, 82–85]. Interestingly, hantavirus infected LECs are also hyperresponsive to VEGF and hypoxia and activate mTOR signaling responses that are inhibited by rapamycin as well as VEGF-C, which exclusively acts on LEC VEGFR3 receptors [42, 86, 87]. These findings link hantavirus pathogenesis to LEC receptor usage and further suggest a role for hantavirus infection of LECs as a determinant of fluid accumulation within HPS patients. Furthermore, these results suggest that VEGF permeability responses contribute to vascular permeability and clearance deficits and are potential targets for therapeutic intervention [88–93].

Therapeutic regulation of these responses appears to go hand in hand with the receptor and pathway specific regulation of VEGFR2-, Src-, and mTOR-directed responses that control EC barrier functions. Antibodies to VEGF suppress EC permeability and have the potential to antagonize VEGR2 signaling pathways as a means of reducing acute pulmonary edema in HPS [14, 27, 74, 81, 94–101]. In addition, well studied VEGFR2 and Src inhibitors, the mTOR inhibitor rapamycin, and other components which target intermediary steps in EC pathway activation are in clinical trials for treating human cancers but also have the potential to reduce the severity of viral EC permeability-based diseases [28, 81, 94–98, 101–103]. These include Ang-1, S1P, and the drugs pazopanib and dasatinib. Angiopoietin-1, an EC specific growth factor, binds Tie-2 receptors and blocks VEGFR2 directed permeability [100, 104–109] and S1P, a platelet derived lipid mediator, stabilizes vascular barrier functions through Edg-1 receptor signaling [74, 95, 96, 110–113]. The redundant regulation of EC barrier functions provides several mechanisms by which EC receptors may contribute to permeability deficits, but also provides a target rich environment for restoring MEC barrier and LEC fluid clearance functions during viral infection. Understanding these receptor and pathway specific mechanisms is likely to provide a means for resolving viral hemorrhagic and edematous diseases by therapeutically targeting EC responses.

Conclusions

These studies highlight important cell surface targets that have the potential to regulate virally induced vascular permeability and for which there currently are clinically available therapeutics. Targeting EC responses may be broadly applicable to counteracting the severity of additional viral infections that disrupt normal endothelial cell function.

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** Outstanding references with seminal findings that provide a basis for understanding microvascular and lymphatic vessel permeability and edema regulation, VEGF as a primal permeability factor and the endothelium as a potential target for regulating virally induced vascular leakage.

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Highlights (for review)

- Viruses that nonlytically cause vascular leakage infect the vascular & lymphatic endothelium.
- Dengue infected ECs elicit immune enhancing chemokines and direct immune cell targeting of ECs.
- Hantaviruses increase vascular permeability of AJs in response to VEGF signaling in MECs and LECs.
- The endothelium is a therapeutic target for resolving hemorrhagic and edematous disease.

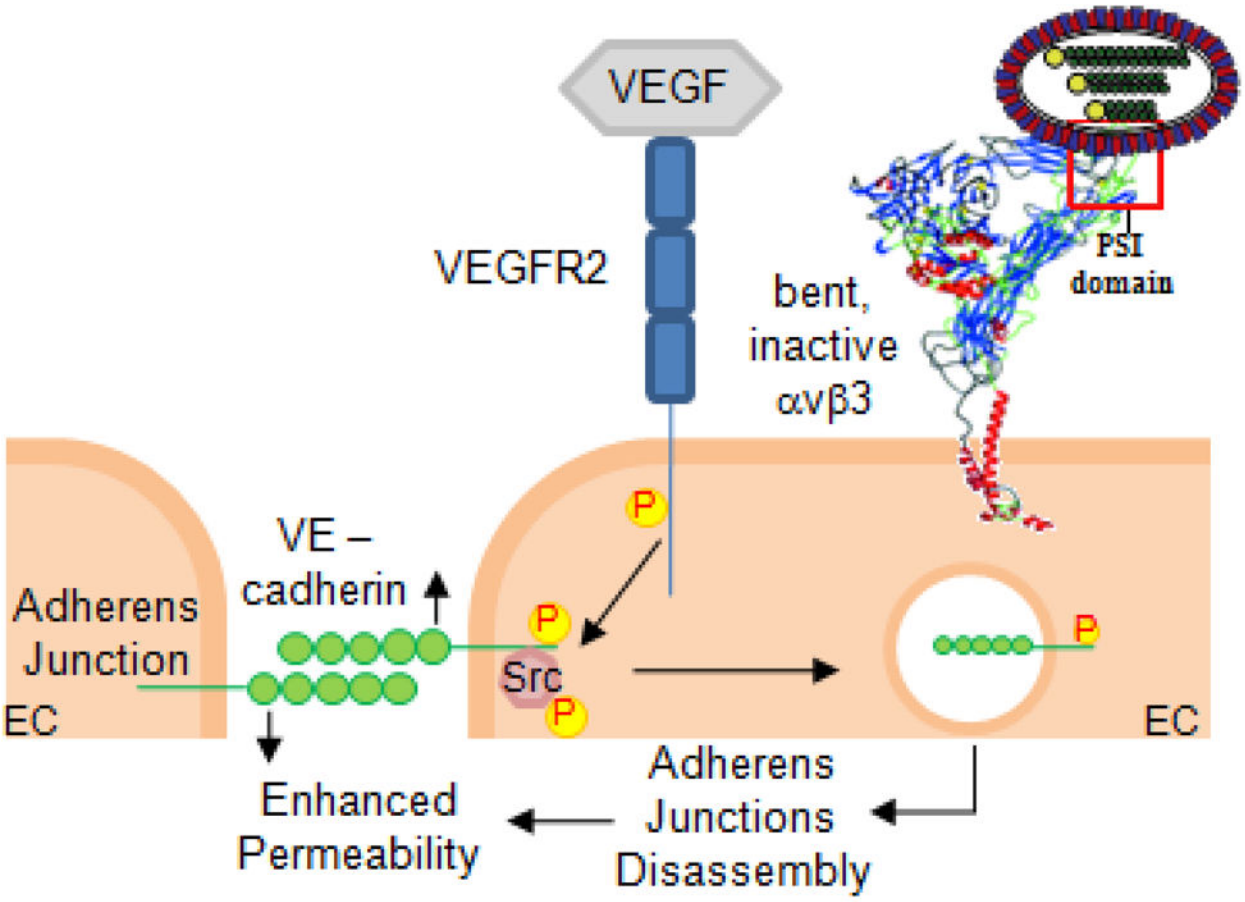

Figure 1.

The figure depicts the inability to $\alpha_v\beta_3$ to regulate endothelial cell responses to hypoxia or VEGF at late stages of hantavirus infection resulting a localized increase in vascular permeability [7, 43, 79, 81, 86, 114]. $\alpha_v\beta_3$ integrins form an extracellular complex with VEGFR2 receptors that normally restrict the permeabilizing effects of VEGF [115–118]. The schematic indicates the effect of pathogenic hantavirus binding to inactive $\alpha_v\beta_3$ integrin conformers [79]. Consistent with hantavirus dysregulation of $\alpha_v\beta_3$-VEGFR2 responses, days after infection cell associated hantavirus coats the surface of endothelial cells [80, 119]. Hypoxia induced VEGF responses of HPS patients [120–123] are likely to enhance VEGFR2-Src signaling responses, which direct VE-cadherin internalization and dissociate adherens junctions (AJs) [7, 42, 43, 81, 86, 114]. VE-cadherin internalization decreases fluid barrier functions of the endothelium [97, 98] and may contribute to localized increases in vascular permeability [17, 29, 124] and edema during hantavirus infection [6, 18, 89, 121, 125]. Thus dysregulation of $\alpha_v\beta_3$ functions may contribute to the enhanced VEGF responsiveness and permeability of hantavirus infected lymphatic and microvascular endothelium.