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Vascular permeability to plasma, plasma proteins, and cells: an update

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Abstract

Purpose of review—The blood vasculature supplies tissues with nutrients, clears waste products, and carries and directs leukocytes to inflammatory sites. To accomplish these functions, microvessels regulate the extravasation of small molecules, plasma proteins and inflammatory cells. The mechanisms responsible for these events have been the subject of intense investigation and, often, dispute.

Recent findings—Recent progress has contributed to a better understanding of the mechanisms by which microvessels of different types and in different vascular beds regulate the passage of small and large molecules and cells. Roles are shown for the glycocalyx, caveolae, pericytes, S1P, and newly discovered signaling pathways.

Summary—Vascular permeability is important for maintaining homeostasis and is greatly increased in acute and chronic inflammation, wound healing, and growing tumors. New work has contributed importantly to the mechanisms responsible for regulating permeability.

Keywords

Paracellular and transcellular permeability; glycocalyx; caveolae; caveolin; VEGF; S1P

Introduction

Vascular permeability takes place by *paracellular* (intercellular) and/or *transcellular* routes and occurs in three distinct contexts [1]: **i.** *Basal vascular permeability* (BVP) refers to the rapid, *paracellular* flux of small molecules (water, salts) and the very limited *transcellular* passage of plasma proteins across normal capillaries. **ii.** *Acute vascular hyperpermeability* (AVH) refers to the extensive but time-limited passage of plasma and plasma proteins across post-capillary venules by either *paracellular* or *transcellular* routes in acute inflammation. And **iii.** *Chronic vascular hyperpermeability* (CVH) refers to the extensive extravasation of plasma and plasma proteins from the angiogenic “mother” vessels of tumors, healing wounds and chronic inflammation [2]). In all cases, the barrier that must be crossed includes EC, vascular basement membranes and pericytes. Vascular beds in different tissues may respond differently to different agonists.

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The mass of plasma solvent and solutes that crosses the vascular wall depends on three different factors [*3]: **i.** Pressure and concentration gradients. **ii.** Hemodynamic forces (blood flow, vascular area available for exchange). And **iii.** The intrinsic permeability of the vascular wall. Assays such as the Miles assay measure the net effect of all three factors, i.e., the flux of solute, often plasma albumin, which extravasates from the mix of different blood vessel types present in tissues. In contrast, intrinsic permeability is determined by measuring several coefficients in individual cannulated blood vessels: L_p , hydraulic conductivity, essentially the ease with which water extravasates; σ , the reflection coefficient, the fraction of a solute (e.g., albumin) that cannot be dragged across the vessel wall by the convective flux of solvent; and P_s , the degree to which the vessel wall restricts the diffusion of a solute.

Discussed below is recent progress in our understanding of vascular permeability under the headings of glycocalyx, caveolae/caveolin-1, S1P, and signaling mechanisms.

EC Glycocalyx

There has been longstanding controversy as to whether the structure referred to as the EC glycocalyx exists. Newer methodologies have now confirmed earlier electron microscopic studies, demonstrating that the glycocalyx does exist and has functional importance for vascular permeability. The glycocalyx is an up to 1 μ m thick coating of the apical surface of normal vascular endothelium and provides the EC-blood interface. Glycocalyx is comprised of transmembrane proteins, predominantly syndecan-1 and glypicans, and their associated heparan sulfate and chondroitin sulfate side chains.

Chappell et al [*4,5] have demonstrated that shedding of the glycocalyx induced by the inflammatory cytokine TNF- α or by ischemia-reperfusion causes a substantial increase in vascular permeability to both plasma and colloids. Loss of glycocalyx could be prevented by either antithrombin III or hydrocortisone. In another study, Potter et al [6] degraded the glycocalyx of mouse cremaster microvessels with glycosaminoglycan-digesting enzymes or with TNF- α . Once removed, recovery of the hydrodynamically relevant glycocalyx required 5–7 days. However, glycocalyx was almost entirely absent from cultured EC and could not be generated, even after prolonged culture, although EC produced all of the major glycocalyx constituents.

In related work, Salmon et al [*3] investigated the mechanisms by which Ang-1, a ligand for the tyrosine kinase receptor Tie-2, affected BVP. They found that Ang-1 reduced L_p and increased σ in normal mesenteric vessels of frogs and rats lined by continuous endothelium and reduced the L_pA of vessels with fenestrated endothelium. Pronase digestion of glycocalyx caused a substantial increase in L_p that was mitigated by pre-perfusion with Ang-1. Also, perfusion with Ang-1 nearly doubled glycocalyx thickness. Together, these studies indicate that Ang-1 reduces BVP to a considerable extent by affecting the glycocalyx and so the coefficients that regulate intrinsic permeability.

Caveolae and its primary structural protein, cav-1

Caveolae were first described by Palade in capillary EC as 50–100 nm in diameter, smooth membrane-bound cytoplasmic vesicles [7]. Palade proposed that caveolae shuttled across capillary endothelium, carrying with them “cargoes” of plasma, and so provided the tissues with the plasma proteins needed for maintaining homeostasis. Paradoxically, several laboratories had reported that plasma albumin was cleared more rapidly in cav-1^{-/-} than in wt mice [see *8].

Recent work from several laboratories has advanced our understanding of the roles of caveolae and cav-1 in regulating vascular permeability. It has long been known that when PMN and

other circulating leukocytes bind to and traverse vascular endothelium, plasma and plasma proteins also leak. The Malik laboratory [*9] investigated the mechanisms responsible for PMN-induced vascular hyperpermeability. PMN activated by phorbol ester released mediators (oxidants, proteases) that disrupted interendothelial junctions and so increased *paracellular* permeability. In contrast, PMN activated by fMLP increased albumin *transcytosis* across rat lung microvasculature *without* opening EC junctions. Several steps were involved: binding of PMN to EC; ICAM-1 clustering; Src activation; and Src phosphorylation of cav-1. Albumin transcytosis could also be enhanced without PMN by crosslinking ICAM-1; further, anti-ICAM-1 antibodies blocked albumin endocytosis and *transcytosis*, as well as Src and cav-1 phosphorylation. Knockdown of cav-1 also prevented PMN-induced albumin transcytosis.

These studies were extended to albumin transport in vivo. Perfusion of isolated rat lungs with fMLP-activated PMN greatly induced albumin hyperpermeability and pulmonary edema; in contrast, transport of mannitol, a low molecular weight substance that traverses the endothelial barrier by the *paracellular* pathway, was not affected. Perfusion with filipin, a cholesterol-binding agent that ablates caveolae, blocked the increased transport of albumin induced by activated PMN. Administration of cav-1 siRNA also significantly depleted total lung cav-1 expression and caused a significant decrease in basal as well as in PMN-stimulated albumin PS. The rapidity with which the siRNA knocked down cav-1 protein activity is surprising. In cav-1^{-/-} mice, basal albumin PS was reduced and activated PMN did not increase albumin PS. Studies with sepsis-induced ARDS are consistent with these findings in that patients commonly develop extensive extravascular fluid accumulation with intact EC junctions.

PMN express cav-1 but at much lower levels than EC [*10]. Nonetheless, PMN cav-1 was important in regulating endothelial permeability. PMN from cav-1^{-/-} mice attached to and traversed EC monolayers less effectively than their wt counterparts. In vivo, fMLP- and PAF-stimulated PMN from cav-1^{-/-} mice were much less effective than wt PMN in inducing permeability to solute and in crossing the lung endothelial barrier.

Chang et al [*8] investigated the role of caveolae, cav-1 and VVOs in AVH and CVH. VVOs are clusters of hundreds of variably sized, interconnected vesicles and vacuoles that extend across normal venular EC cytoplasm from lumen to ablumen; in response to permeabilizing agents such as histamine or VEGF, they open to provide a *transcellular* route for plasma protein extravasation in wt mice [2,11]. As expected, caveolae-sized vesicles were strikingly reduced in the capillary and venular endothelium of cav-1^{-/-} mice [*8]. Further, nearly all capillary caveolae and similar-sized vesicles in venular endothelium of wt mice labeled with anti-caveolin antibodies, but only 1/3-1/2 of larger vesicles and vacuoles in VVOs were so labeled. Nonetheless, VVOs were found in equal numbers and with indistinguishable composition in the venular endothelium of cav-1^{-/-} and wt mice. However, VEGF-induced AVH was strikingly reduced in cav-1^{-/-} mice, despite the presence of normal appearing VVOs. This finding suggests that, although caveolin-1 is not essential for VVO formation, it is important for VVO function.

These authors also found that transformation of venules into hyperpermeable mother vessels, the initial step in VEGF-induced angiogenesis [2], is strikingly reduced in cav-1^{-/-} mice [*8]. Mother vessel formation was shown to involve digestion of venular vascular basement membranes by pericyte cathepsins, coupled with a simultaneous decrease in cathepsin inhibitors; with loss of basement membrane, pericytes detached and EC thinned and expanded to cover an enlarged surface area [*12]. Venular VVOs contributed to this process by donating their membranes to the rapidly expanding plasma membrane. Thus, the failure of mother vessels to form in cav-1^{-/-} mice in response to VEGF or tumors likely reflects the absence of cav-1 in VVOs, indicating that cav-1 has an important role in VVO function in both AVH and CVH.

Sanchez et al [13] used genetically modified eNOS and cav-1 mice to investigate the mechanisms of AVH. They found that eNOS endocytosis by caveolae to an as yet undetermined subcellular target is a necessary step for NO production and so for the increased AVH induced by PAF and VEGF.

Sphingosine-1-Phosphate (S1P)

S1P is an important regulator of post-capillary venule permeability. It acts through two receptors, S1P₁ and S1P₂, which are differentially expressed in different vascular EC beds and can mediate antagonistic activities. Skoziura and Hla [14] have recently reviewed S1P, but I summarize here several newer advances. Zhao et al [15] demonstrated that bone marrow-derived progenitor cells (BMPC) from wt mice acted through the S1P pathway to enhance endothelial barrier function and prevent increased vascular permeability, edema formation and lethality in phorbol ester-challenged mice. BMPC from sphingosine kinase null mice failed to do so, implicating S1P and S1P₁. Whereas HUVEC primarily express S1P₁, cremaster muscle EC express both S1P₁ and S1P₂ [*16]. As a result, S1P did little to suppress histamine-induced permeability in cremaster muscle as S1P activated both receptors whose actions are antagonistic. Similarly, Camerer et al [*17] generated mice lacking plasma S1P. These mice exhibited greatly increased BVP (lungs > paws) as well as greater AVH to histamine; further, passive systemic anaphylaxis was considerably more lethal than in wt mice. Of interest, these mutant mice could be rescued by transfusion of red blood cells, which express sphingosine kinases.

A potpourri of signaling papers

The signaling pathways by which different agonists modulate vascular permeability is a subject of enormous complexity. Significant progress was made in the last year on many different fronts and results from selected papers are presented below.

- Pericyte secreted TGF- β has long been known to participate in vessel stabilization *in vitro*. Walshe et al [*18] investigated the role of TGF- β in the retinal vasculature. TGF- β interacts with its receptor, TGF β RII, to recruit and phosphorylate TGF β RI, with subsequent downstream recruitment of smad transcription factors. TGF- β signaling was inhibited by systemic expression of endoglin (TGF β RIII), with a resulting breakdown of the blood-retinal barrier and consequent increased *paracellular* permeability. EC express two TGF β RI receptors, ALK5 and ALK1. Signaling through ALK5 stabilizes vascular endothelium, whereas signaling through ALK1 limits ALK5 signaling and causes vascular destabilization. These findings help to elucidate the signaling mechanisms by which pericytes maintain the integrity of the normal retinal microvasculature.
- The neurotransmitter dopamine was shown some years ago to inhibit VEGF-A-induced CVH and angiogenesis by inhibiting phosphorylation of VEGFR-2 [19]. Sinha et al [*20] have now worked out some of the signaling mechanisms. They demonstrated that the dopamine receptor, D2DR, normally co-localizes with VEGFR-2 at the EC surface. On addition of VEGF, SHP-2 translocated from the cytosol to the cell surface and associated with both VEGFR-2 and D2DR. VEGF promoted the dissociation of VEGFR-2 from D2DR and induced VEGFR-2 phosphorylation and downstream signaling. Dopamine pretreatment prevented VEGFR-2 internalization, maintaining the association of D2DR with VEGFR-2. Dopamine also increased the association between SHP-2 and D2DR at the cell surface and stimulated the phosphorylation of SHP-2 and its phosphatase activity. Active SHP-2 inhibited VEGFR-2 phosphorylation at Y951, Y996 and Y1059, but not at

Y1175. Decreased phosphorylation of VEGFR-2 at Y951 decreased Src phosphorylation at Y418 and its kinase activity, thus inhibiting VEGF activity.

- Knezevic et al [*21] investigated the signaling pathways by which PAF increases *paracellular* permeability. PAF interaction with its receptor activated the Rho GTPase, Rac1. Rac1 and its guanine nucleotide exchange factor, Tiam1, became associated with a membrane fraction from which they co-immunoprecipitated with the PAF receptor. Simultaneously, actin polymerized to form stress fibers, the junctional proteins ZO-1 and VE-cadherin relocated from interendothelial junctions, and inter-EC gaps formed *without* cell contraction or activation of RhoA. The response was independent of myosin light chain phosphorylation, and therefore distinct from the permeability responses induced by other agents such as histamine and thrombin.
- Ramachandran et al [*22] investigated the role of Cdc42 in maintaining BVP in lungs. They generated transgenic mice that expressed the dominant active mutant V12Cdc42 protein selectively in EC. These mice demonstrated a markedly reduced increase in lung microvascular permeability in response to lipopolysaccharide, compared with wt mice. Thrombin-induced transendothelial electrical resistance, a measure of open interendothelial junctions, was reduced in monolayers of EC expressing the V12Cdc42 mutant, although baseline permeability levels were unchanged. RhoA activity was also reduced compared with wt EC, suggesting that Cdc42 functions by counteracting the canonical RhoA pathway of endothelial hyperpermeability.
- Activated protein C (APC) has been shown to reduce mortality in sepsis patients [23]. Using both *in vivo* and *in vitro* models, Schuepbach et al [*24] demonstrated that this effect was mediated by APC cleavage and thus activation of the thrombin receptor PAR-1. Pharmacological concentrations of APC had powerful barrier protective effects on cultured murine EC monolayers that were mediated through PAR-1 cleavage. Endogenous overexpression or intravenous injection of APC inhibited permeability induced by VEGF in the Miles assay and also protected against pulmonary edema in endotoxemia. As predicted, APC did not significantly alter the vascular barrier function in PAR1-deficient mice.
- The Sessa lab found that AVH was reduced in Akt-1^{-/-} mice [25]. In cultured EC isolated from Akt-1^{-/-} mice, histamine gave a blunted increase in transendothelial resistance with reduced phosphorylation of VE-cadherin, steps which lie downstream of eNOS. However, inflammatory cells isolated from Akt-1^{-/-} mice responded efficiently to inflammatory stimuli in wt mice, indicating that the permeability defect in Akt-1^{-/-} mice is restricted to EC.
- Thrombospondin-1 (TSP-1) is a known inhibitor of VEGF-induced permeability and angiogenesis and tumor growth is increased in TSP-1^{-/-} mice [26]. Zhang et al [*27] found that the TSP-1 receptors, CD36 and β -1 integrin subunit, form a complex with VEGFR-2. 3TSR (a recombinant protein comprised of the three type-1 repeats in TSP-1 that represent its antiangiogenic domain) suppressed VEGF-induced vascular permeability and angiogenesis by inhibiting phosphorylation of VEGFR-2, both *in vivo* and *in vitro*. Unexpectedly, however, TSP-1 null mice exhibited decreased VEGF-induced phosphorylation of VEGFR-2 *in vivo* and *in vitro* and diminished vascular permeability in response to VEGF.

Conclusion

This paper has presented a subjective summary of progress made during the last year toward an understanding of the mechanisms of vascular permeability. Despite enormous progress, we

still lack a comprehensive picture of the mechanisms by which different agonists induce either *paracellular* or *transcellular* permeability. Space limitations made it necessary to eliminate certain important areas of investigation, and the interested reader is referred to a review by Dejana et al [28] for progress with respect to the control of vascular integrity by endothelial cell junctions.

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Abbreviations

EC	endothelial cells
BVP	basal vascular permeability
AVH	acute vascular hyperpermeability
CVH	chronic vascular permeability
VEGF	vascular endothelial growth factor
PMN	neutrophils
PAF	platelet activating factor
cav-1	caveolin-1
S1P	sphingosine-1-phosphate
TNF- α	tumor necrosis factor-alpha
Ang-1	angiopoietin-1
wt	wild-type
cav-1	caveolin-1
VVOs	vesiculo-vacuolar organelles
VEGFR-2	vascular endothelial growth factor receptor-2
SHP-2	Src-homology-2-domain-containing protein tyrosine phosphatase

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