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Inflammation without vascular leakage – science fiction no longer?

Niall C. Filewod[1,2], Warren L. Lee[1,2,3*]

[1]Department of Critical Care Medicine, St. Michael's Hospital
[2]Keenan Research Centre for Biomedical Sciences, St. Michael's Hospital
[3]Interdepartmental Division of Critical Care Medicine, University of Toronto

*Corresponding author:
Warren L. Lee, MD PhD
St. Michael's Hospital
30 Bond Street,
Toronto, Ontario, CANADA
M5B 1W8
leew@smh.ca

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Abstract

Vascular leakage is a characteristic of critical illnesses such as septic shock and acute respiratory distress syndrome. It results in hypotension and tissue edema and contributes to organ dysfunction. It has long been taught that increased vascular permeability is a natural consequence of inflammation; in particular, many clinicians believe that it occurs inevitably during leukocyte recruitment to a site of infection. In fact, abundant research now indicates that vascular leakage and leukocyte emigration do not necessarily occur together in a blood vessel. The molecular mechanisms underpinning these processes – allowing leukocytes to exit the circulation without increasing vascular permeability – are starting to be elucidated and establish vascular leakage as a viable therapeutic target. Several pre-clinical studies indicate that vascular leakage can be reduced without impairing cytokine production, leukocyte recruitment and pathogen clearance. The realization that leukocyte traffic and vascular permeability can be regulated separately should spur development of therapies that decrease vascular leakage and tissue edema without compromising the immune response.

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Niall C. Filewod, MD and Warren L. Lee, MD PhD

Calor, dolor, rubor, tumor – since Celsus surveyed Western medical knowledge in *De Medicina* almost two millennia ago (1), clinicians have recognized tissue swelling and vascular leakage as a cardinal sign of inflammation. As an understanding of the circulatory system developed, the need for leukocytes to exit the vasculature to fight infection became central to an early debate over the existence of capillaries. Some argued that blood must perfuse tissues like a stream in a field, without distinct capillary walls – otherwise, how could leukocyte emigration from the circulation occur (2)? In fact, all blood vessels, even the capillaries and post-capillary venules where most leukocyte emigration occurs (3), are lined by a layer of endothelial cells which made cell trafficking from blood to tissue seem paradoxical in the absence of endothelial damage. In 1844, a writer in *The Lancet* remarked: "It is impossible that corpuscles of the blood can pass through it (the capillary membrane) without rupture" (cited in (2)).

To some degree, this view persists even today: many clinicians still believe that vascular leakage is a necessary consequence of leukocyte recruitment from the blood into the tissue. How could circulating leukocytes traverse the underlying endothelium without causing increased endothelial permeability? A logical extension to this belief is the fear that trying to *prevent* vascular leakage will suppress leukocyte emigration and will therefore be harmful to the immune response.

Research is challenging this widely-held view. Numerous investigators have now shown that vascular leakage and leukocyte emigration often occur separately in the same blood vessel (4, 5). Advances in intravital microscopy, cell biology and molecular biology have challenged the notion that vascular leak is required for leukocyte trafficking (6, 7); *how* this distinct regulation occurs is now being elucidated. In post-capillary venules, adjacent cells are held together by adherens junctions, of which vascular endothelial (VE)-cadherin is the principal component (8). Adherens junctions hold the capillary monolayer together and interact with each endothelial cell's actin cytoskeleton, a scaffold that underpins cellular structure. Most leukocyte trafficking out of the circulation occurs "paracellularly", or *between* adjacent endothelial cells – with leukocytes 'unzipping' the endothelial cell-cell junctions.

How this disassembly of cell-cell junctions occurs without causing leakage of plasma and proteins is starting to be understood. While leukocyte emigration requires dephosphorylation of a specific tyrosine residue on VE-cadherin, vascular leakage in response to inflammatory mediators such as histamine requires the phosphorylation of another, distinct tyrosine residue (9) (Figure 1). Furthermore, the size of the endothelial pore that allows leukocytes to leave the circulation is tightly regulated by a GTPase enzyme called RhoA; RhoA controls actin dynamics in the endothelial cell, essentially drawing the cell body tight like a drawstring around the emigrating leukocyte (10). Other studies have shown that endothelial cells limit fluid leakage during leukocyte emigration by forming actin-dependent "domes" over the site of the migrating leukocyte – a sort of "airlock" that keeps vascular disruption to a minimum (11, 12). In some circumstances, rather than creating a pore between cells, leukocytes are able to traverse the cytoplasm of *individual* endothelial cells – a relatively poorly understood phenomenon known as

transcellular diapedesis (13). Remarkably, whether leukocytes emigrate paracellularly or transcellularly, actin-dependent domes seem to prevent excess vascular leakage (11) (Figure 2). Many questions remain, but it is clear that leukocyte trafficking and vascular leakage *can* be regulated separately, meaning that one is not the inevitable consequence of the other. In our opinion, this fact is almost completely unappreciated among clinicians and even among many researchers in this field.

What do these findings mean for clinicians? Increased vascular leakage is a hallmark of critical illnesses such as septic shock (14) and acute respiratory distress syndrome (ARDS) (15). But rather than simply being a symptom of inflammation, vascular leakage leads to intravascular volume depletion and hypotension. The resulting therapeutic administration of fluids exacerbates tissue edema which may contribute to organ failure (16). The accumulation of extravascular fluid might impair organ function by impeding the diffusion of oxygen or by increasing interstitial pressure, compromising microvascular perfusion. In the brain, cerebral edema can raise intracranial pressure and induce cerebral ischemia and frank herniation; its avoidance or amelioration has long been a therapeutic goal (17). There are also emerging data to suggest that vascular leakage may predispose to tissue fibrosis, making it relevant not only to acute syndromes like ARDS but also to more chronic diseases like systemic sclerosis (18) and chronic kidney disease (19).

The development of medications to reduce vascular leakage has been dogged by concern that this might impair the immune response and affect pathogen clearance. Thus, the recognition that leakage and cell trafficking are distinct processes suggests an area of unmet therapeutic opportunity. Although the field is young, there are already numerous preclinical reports of potential therapeutic agents that – by different mechanisms - inhibit vascular leakage and edema without impairing innate immunity (Table 1). For example, modulation of the angiopoietin–Tie2 signalling axis (20) improved survival in mouse models of intra-abdominal sepsis (21) and influenza-induced ARDS (22). These studies – which used a novel peptide to activate the Tie2 receptor – showed that improvement in vascular integrity was achieved without impairment in pathogen clearance and, in two recent ARDS studies, without affecting alveolar neutrophil recruitment or cytokine levels (22, 23). As a second example, enhancement of vascular integrity using the Slit2N protein improved survival in murine models of sepsis and ARDS with no defect in pathogen clearance or cytokine production; Slit2N decreased vascular leakage by increasing retention of VE-cadherin at the endothelial plasma membrane (24). Recently, a third group has reported that antibody-blockade to β1-integrin significantly improved vascular leakage in murine endotoxemia without affecting alveolar neutrophil recruitment or endothelial cell activation (25). The protective effect was attributed to blocking actin stress fiber formation and endothelial cell contraction. Finally, a fourth group has described a role for the GTPase ARF6 in regulating internalization of VE-cadherin and endothelial permeability (26); the same investigators showed that inhibition of ARF6 reduced vascular leakage and improved survival from murine endotoxemia without affecting serum cytokine levels (27).

In these studies, the fact that elevated levels of circulating cytokines were not perturbed by enhancement of vascular integrity supports the notion that leakage can be regulated distinctly from the inflammatory response. These observations, which

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emanate from multiple groups and use agents acting via multiple mechanisms, clearly establish the feasibility of reducing vascular leakage without impairing innate immunity.

There is considerable literature on other agents or approaches that reduce vascular leakage (reviewed in (28)) – prominent examples include adrenomedullin (29-31), inhibition of vascular endothelial-protein tyrosine phosphatase (VE-PTP)(32, 33), inhibition of Abl kinases with imatinib (34-36), sphingosine-1-phosphate (37, 38) – with the caveat that most of these have also been reported to inhibit leukocyte transmigration or other aspects of innate immunity. In some cases, modulation of the influx of leukocytes in addition to a reduction in edema may seem desirable – for instance, to counteract the delayed apoptosis of parenchymal neutrophils that is thought to contribute to tissue injury (39). However, inhibition of innate immunity and the resultant immunosuppression remain a concern. For instance a classic scenario is the development of post-influenza bacterial pneumonia, one of the commonest causes of death after pandemic influenza virus infection (40). In the setting of influenza-induced acute lung injury, immunosuppression could be detrimental but the ability to decrease vascular leakage without affecting leukocytes would be particularly attractive.

In closing, it is our hope that the recognition that decreasing vascular leakage need not impair leukocyte trafficking or pathogen clearance will encourage the development of therapeutics that modulate endothelial permeability while preserving the immune response. We anticipate that further delineation of the underlying mechanisms will stimulate rapid growth in this nascent field. In addition to obvious applications to septic shock and ARDS, such agents could have broad utility in disorders ranging from stroke to ischemia-reperfusion injury after cardiac arrest. The prospect of preventing or treating tissue edema without compromising the immune response should no longer be considered science fiction.

Acknowledgements

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Figure Legends:

Figure 1. Distinct regulation of vascular leakage and leukocyte emigration can be explained in part by selective phosphorylation and dephosphorylation of specific tyrosine residues on VE-cadherin, the major constitutent of intercellular adherens junctions. In this schematic, stimulation with histamine or vascular endothelial growth factor (VEGF) induces phosphorylation of tyrosine 685 and results in vascular leakage. In contrast, leukocyte adhesion stimulates dephosphorylation of tyrosine 731 and is required for emigration.

Figure 2. Another mechanism that limits vascular leakage during leukocyte diapedesis involves remodelling of the endothelial actin cytoskeleton. This results in the formation of a dome-like structure above the emigrating leukocyte as well as the formation of a tight-fitting pore around the leukocyte. The latter has been shown to require the GTPase RhoA.

Table 1. Selected strategies for decreasing vascular leakage and their effect on innate immunity

Agent/Strategy	Mechanism of action to decrease vascular leakage	Effect on innate immunity
Inhibition of ARF6 (26, 27)	Decreases internalization of VE-cadherin; regulates VEGFR2 trafficking	No decrease in cytokine production during acute inflammation (murine arthritis model); pathogen clearance not reported
Slit2N (24)	Decreases internalization of VE-cadherin	No decrease in systemic or lung cytokine levels in murine models of sepsis and ARDS; no effect on pathogen clearance
Vasculotide (22-23)	Activates Tie2 receptor	No decrease in cytokine production in murine models of ARDS; no effect on pathogen clearance; no effect on alveolar neutrophil recruitment
Anti-β1-integrin antibodies (25)	Prevents stress fiber formation and endothelial cell contraction	No effect on serum cytokine levels or alveolar neutrophil recruitment in murine endotoxemia
Adrenomedullin (29,30,31)	Decreased actin stress fiber formation via increased cAMP	Decreased alveolar leukocytes but not cytokines in a murine model of ventilator-induced lung injury
Inhibition of VE-PTP (32,33)	Activation of Tie2 signaling and stabilization of the actin cytoskeleton via FGD5	Decreased alveolar neutrophil transmigration in response to nebulized endotoxin in mice
Imatinib (34, 35, 36)	Inhibition of Abl kinases and enhancement of cell adhesion	Decreased alveolar neutrophils and cytokines in a murine model of lung injury
Sphingosine-1-phosphate (37, 38)	Rac activation and cytoskeletal remodeling; cell-cell junction assembly	Decreased leukocyte migration in numerous models

This list and the accompanying citations are not intended to be comprehensive. Abbreviations: VEGFR2: a receptor for vascular endothelial growth factor; VE-PTP: vascular endothelial-protein tyrosine phosphatase
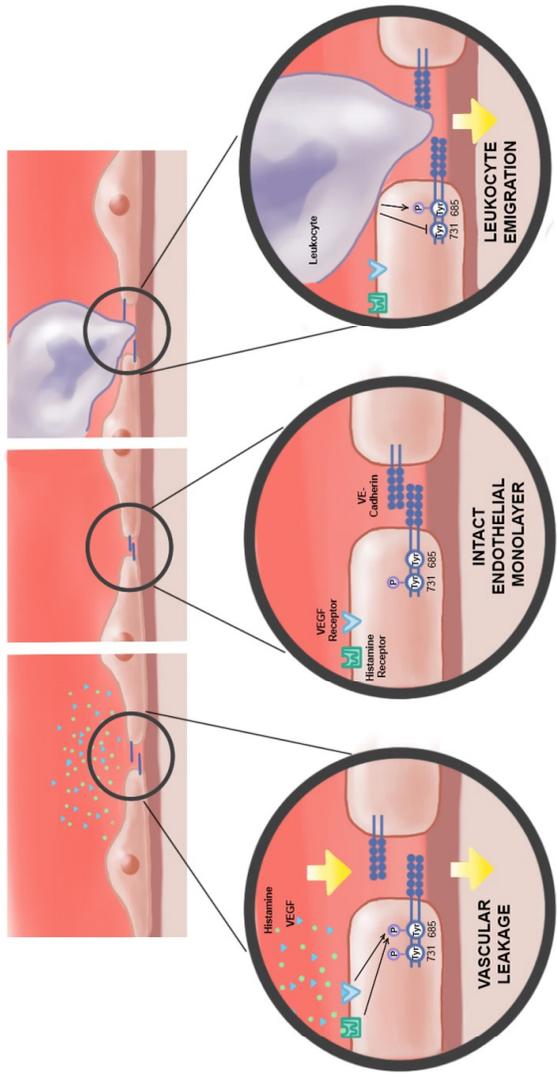
Figure 1

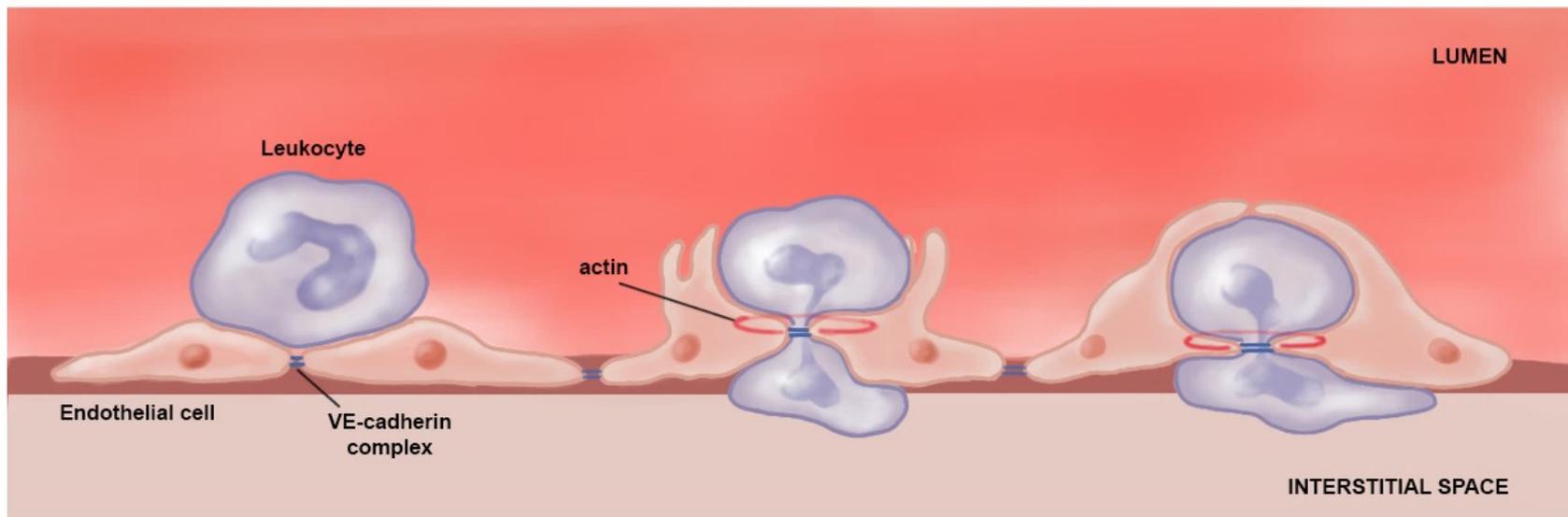**Figure 2**