Spotlight on microvascular permeability

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This editorial refers to a collection of fourteen review articles invited for this special issue on microvascular permeability, guest edited by Fitz-Roy Curry and Thomas Noll.

1. Introduction

The normal endothelium forms a stable anti-inflammatory, anti-thrombotic, and anti-adhesive interface between circulating blood components and cells within all tissues of the body. The endothelium and its associated structures, e.g. glycocalyx, basement membrane, and pericytes, form the primary barrier to water and plasma protein exchange. This barrier is sufficient to maintain the plasma volume and venous return, and prevent tissue oedema while it enables transvascular exchange to meet metabolic and homeostatic demands of the tissues. Under normal physiological conditions, an increase in the amount of nutrient exchange is most probably the result of vasodilation to increase the surface area and the driving forces for water (microvessel pressure) and solute exchange (concentration differences) without a significant increase in permeability.¹² The view of the endothelium as a stable barrier allowing selective exchange of water, nutrients, and plasma proteins stands in contrast to the other well-recognized role of the endothelial barrier as a major player in inflammatory processes in which the components of the normal barrier, including the surface glycocalyx and the junctions between adjacent endothelial cells, are acutely or permanently modified as part of immune responses and, at the same time, compromise barrier function. In this case, vasodilation caused by inflammatory mediators or clinical interventions only exacerbates the dysfunctional state. Thus, microvascular exchange is modulated not only by changes in the conductance of the endothelial barrier (measured as a real change in microvascular permeability) but also by local perfusion conditions. The reviews covered in this Spotlight Issue provide a broad survey on many fundamental aspects of the control of microvascular exchange processes under both physiological and pathological conditions.

Although hyperpermeability and oedema formation have been factors long recognized to exacerbate chronic disease states, therapeutic strategies for targeted interventions are rare. One major problem is the lack of diagnostic tools to identify hyperpermeability at an early stage and to follow the changes during attempted therapy. In recent years, magnetic resonance imaging (MRI) techniques to measure the changes in water content have allowed the visualization of local oedema and dynamic changes in water content in the myocardium as well as in other tissues.¹ However, targeted therapeutic interventions are hampered by the fact that MRI techniques, in their present state of development, do not uniquely distinguish the changes in tissue water content that are due to an increased permeability. Nevertheless, we suggest that interventions based on a combination of continued growth in knowledge of the control of microvascular permeability with new MRI imaging as reviewed in this Spotlight Issue will lead to new options for therapeutic intervention to protect against the results of acute or chronic hyperpermeability.

2. Microvascular fluid exchange: new concepts of fluid exchange in normal and high-permeability states

The review by Levick and Michel³ highlights the most fundamental change in our understanding of transvascular fluid exchange over the past years. Although the Starling principle governing fluid exchange across endothelial barriers has been known for more than a century,³ the new understanding of the micromechanics of fluid movement through fenestrated capillaries and the endothelial glycocalyx in continuous capillaries requires modification of the application of the traditional form of the Starling principle. Levick and Michel show how standing gradients of plasma protein within the intercellular cleft and across the glycocalyx in continuous capillaries and around fenestrations can rapidly adjust so that there is slow, steady-state filtration along a microvascular bed, even on the venular side. Steady-state re-absorption in venular microvessels previously assumed to partially balance filtration of the arterial side of the microvasculature cannot occur, except in special cases. Whereas Levick and Michel focus on the balance of hydrostatic and oncotic pressures, the review by Reed and Rubin⁴ highlights active modulation of interstitial pressure by interstitial fibrocytes, which may release tension and allow the underhydrated glycosaminoglycans to swell, take up fluid, and cause large, transient reductions in interstitial pressure. The amount of fluid exchanged may be much larger than expected from the measured hydraulic conductivity and the commonly assumed range of interstitial pressure. These two reviews highlight areas for further research. Loss of the glycocalyx in the region of a discontinuity in...
the tight junction strands between adjacent cells can form localized non-selective regions (large pores) with no widening of normal intercellular junctions. Very large increases in filtration from the normal, steady-state condition can occur in the presence of such large pores.4 Also, the glyocalyx is often not present or ignored in cultured endothelial cells, and ways to preserve and modify this structure to help in understanding the mechanism controlling protein synthesis and turnover are needed. Further, active contractile mechanisms to modify interstitial pressure may be just as important as contractile mechanisms to modify the hydraulic conductivity of the endothelium.

3. Endothelial heterogeneity and methods to measure microvascular permeability in genetically modified mice

Transgenic mouse models represent a powerful new tool to evaluate the results of modulation of endothelial permeability in vivo. Curry and Adamson5 describe methods to measure microvascular permeability in multiple organs, each with different basal permeability properties and differential response in terms of changes in permeability and microvascular perfusion when exposed to modulators of barrier function. These authors also extend the theme of endothelial phenotype heterogeneity, describing examples of endothelial cell phenotype plasticity where the endothelial cells in a single microvessel or microvascular bed change from a normal venular phenotype to a more inflammatory phenotype after exposure to injury or acute inflammation. There is growing evidence that the endothelial phenotype expressed in some cultured endothelial cells is more characteristic of this proinflammatory state than of normal endothelium. The topic of endothelial heterogeneity is expanded further by Huxley and Wang6 who evaluate the differences in microvascular function where gender is likely to be important. These reviews point to the need for further understanding of endothelial cell heterogeneity and phenotype plasticity.

4. Inflammatory mediators and signalling in endothelial permeability regulation

During recent years, studies in cultured endothelial cells have provided an enormous amount of information about the endogenous endothelial mechanisms that can modulate microvascular permeability by altering the adhesion between adjacent endothelial cells or between endothelial cells and their extracellular matrix. Changes in both resting and active tension mediated by the acto-myosin-based cytoskeleton, alterations and adaptation of the cytoskeletal organization, and activation of various forms of transcellular pathways are involved in the control of endothelial barrier function. The reviews by Spindler et al.7 Duran et al.8 Bates,9 and Shen et al.10 highlight the need to evaluate the contribution of different mechanisms to the control of permeability barriers in intact microvascular beds. Bates11 discusses discrepancies between in vitro and in vivo experiments, analyzing the magnitude and time course of the permeability increase as well as the underlying mechanisms, i.e. transcellular vs. paracellular pathways in endothelial cells, after exposure to vascular endothelial growth factor. This review also cites particularly clear examples of the need for critical evaluation of experiments using tissue accumulation of injected tracers such as Evans Blue in a Miles Assay as a reliable measure of changes in microvascular permeability. Both Duran et al.10 and Shen et al.12 describe examples in which transgenic mouse models have pointed the way to new strategies to evaluate the importance of specific mechanisms that increase endothelial permeability in intact animals and activate endothelium-specific myosin light-chain kinase and endothelial NO synthase.

For many years, experiments in cultured endothelial monolayers pointed to endothelial contraction as being the predominant mechanism leading to an increased microvascular permeability, which occurred by rupturing the junctions between adjacent cells. As more was learned about the relative strength of adhesion proteins between endothelial cells in different levels of the microvasculature, arterial vs. venular, the model was modified to describe the regulation of the paracellular pathway (between endothelial cells) in terms of a balance between adhesion and contraction. This model is continually being refined, and the reviews here highlight the current understanding of various forms of this model. Spindler et al.7 update research on the multiple roles of small GTPases that regulate adhesion, cytoskeletal organization, and tension. These small GTPases are points of convergence for signalling pathways initiated by multiple stimuli. Of particular importance is the recent recognition that increased signalling through cAMP, known to enhance barrier function, acts independently of a cAMP-dependent protein kinase (PKA) pathway to activate Rap1 and Rac to stabilize the barrier. Inflammatory mediators may disrupt the barrier, acting at least in part by reducing membrane concentrations of cAMP. Of equal importance is the recognition that one of the mechanisms that contributes to robust increases in permeability is likely to be the up-regulation of Rho A-dependent contractile mechanisms after exposure of endothelium to inflammatory conditions.

5. Leucocyte interactions with endothelium and increased microvascular permeability

It is widely considered that the attachment and migration of leucocytes is synonymous with increased microvessel permeability, but much evidence suggests that there is a distinct dissociation, both spatial and temporal, of sites of leucocyte adhesion and sites of increased vascular permeability. The review by He13 addresses important experiments that dissociate leucocyte adhesion and migration from the action of the products of activated leucocytes, including reactive oxygen species (ROS), to increase the permeability. Most importantly, she reviews experiments that suggest that platelet adhesion to sites where the endothelial barrier is already opened is one of the mechanisms that initiates an inflammatory cascade. This includes not only platelet activation but also the strong interaction of platelets and leucocytes, which results in an enhanced activation of leucocytes. It is of interest that depletion of leucocytes does not attenuate the observed transition to a more inflammatory phenotype in the wound-healing model, but platelet depletion has a significant effect. The above approach uses individually perfused vessels for such dissociation experiments.
The interdependence of leucocyte and platelets is further evaluated by Rodriguez and Granger \(^{14}\) as part of their review on ischaemia/reperfusion injury. The mechanisms involve endothelial cells, leucocytes, platelets, and mast cells as well as tissue macrophages and their products, including cytokines, chemokines, and ROS. Systematic dissection of the components is a complex task, requiring expertise in permeability measurements and studies in cultured cells, isolated vessels, and whole animals. Noteworthy is the use of bone marrow chimeras produced by the transplantation of bone marrow from mice deficient in key enzymes involved in ROS production into wild-type mice to distinguish sources of ROS.

6. The endothelial glycocalyx: regulator of barrier function, perfusion, and mechanotransduction

The reviews on this theme evaluate additional ways in which the endothelial glycocalyx and more loosely attached surface layers may modulate microvascular permeability and other functions of the microvasculature. Becker et al.\(^{15}\) describe conditions where the loss of glycocalyx components compromises normal barrier function and suggest approaches that may preserve the glycocalyx, particularly those strategies that may be useful in the clinic. A similar translational theme is developed by van Teeffelen et al.\(^{16}\) who focus on the ability of the glycocalyx to exclude a range of blood-borne agents and how various agents may modulate this function. Here is evidence that subtle changes in glycocalyx structure may be among the earliest manifestations of impaired endothelial barrier function. This has led the authors of this review to attempt to measure the changes in glycocalyx thickness and volume in various inflammatory diseases in animal and human subjects. The basis for these measurements remains controversial. It is therefore of interest that changes in other glycocalyx-dependent mechanisms, themselves amenable to evaluation in a clinical setting, may also provide early information about the glycocalyx.

An even more direct link between different functions of the glycocalyx is its dual role as a mechanotransducer and determinant of vascular permeability. The structure of the glycocalyx is well suited to detect the shear stress near the vascular wall. Tarbell\(^{17}\) reviews experiments to evaluate the role of the glycocalyx as part of the mechanism involved in shear-dependent increases in permeability after an acute increase in shear stress on cultured endothelial cell monolayers. This action is attenuated by partial removal of the glycocalyx. While there is disagreement about the effect of an acute increase in shear stress on the permeability of intact microvessels, there is increasing evidence that sustained laminar shear is one factor maintaining the stable endothelial phenotype. On the other hand, sustained disturbed flow such as that in the regions of the vascular tree with reverse flow is likely to express a more inflammatory phenotype. The role of the glycocalyx in these processes remains to be evaluated, but there is evidence that the glycocalyx is thinner in these regions.

7. Myocardial microvascular permeability, interstitial oedema, and compromised heart function

The heart is particularly sensitive to increases in microvascular permeability and the accumulation of myocardial interstitial oedema fluid. Heart function is significantly compromised with only a few per cent increase in the interstitial fluid volume. In their review, Dongaonkar et al.\(^{18}\) highlight how microvascular hyperpermeability and myocardial oedema formation, which may occur in response to inflammation or ischaemia/reperfusion, as well as clinical interventions such as cardiopulmonary bypass and cardioplegic arrest common to many cardiothoracic surgical procedures, compromise cardiac function. The authors discuss the acute changes that take place in the myocardium to further compromise cardiac function following oedema resolution, and compensatory changes in the interstitial matrix of the heart in response to chronic myocardial oedema. These are problems where refinement of new MRI approaches to detect interstitial water accumulation may provide important new therapeutic advances.

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References